PATENT ABSTRACTS OF JAPAN

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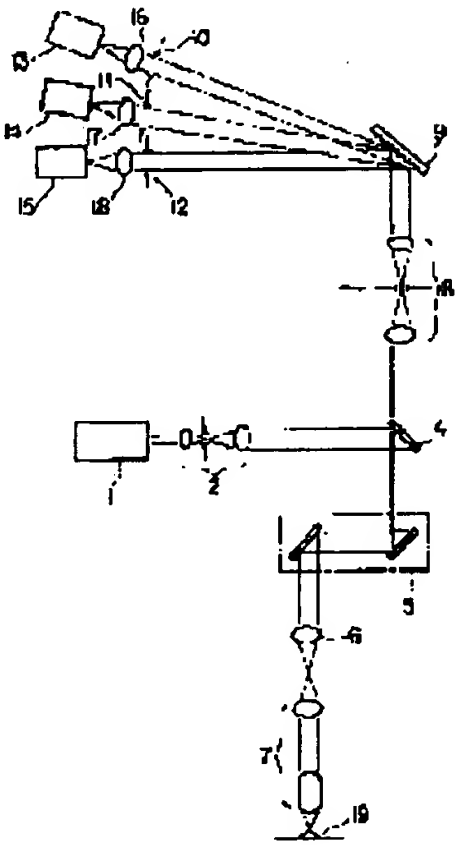
(54) SCANNING OPTICAL MICROSCOPE

(57)Abstract:
PURPOSE: To obtain a scanning optical microscope capable of fluorescence detection excellent in S/N and having no fluorescence crossover at the time of multiple coloring without using a filter or the like having wavelength depen dency as a photometry separation means.
CONSTITUTION: This microscope is equipped with a laser light source means(a laser light source 1, a beam expander 2, a dichoric mirror 4, an XY scanning optical system 5, a pupil projection lens 6 and a microscope 7) for projecting the laser beam of at least single wavelength or more so as to irradiate a sample 19, and the photometry separating mean for guiding the fluorescence emitted from the sample 19 to photodetectors 13, 14 and 15 corresponding to variable width slits 10 to 12 via a grating 9 arranged on an optical path for photometry and single or more variable width slits (10, 11 and 12).



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CLAIMS

[Claim(s)]
[Claim 1] A laser light source means to scan the laser beam of single wavelength at least, and to irradiate a sample, The detection optical system which detects the light from said sample, and the image formation optical system which carries out image formation of the light from said sample, The confocal diaphragm arranged in the focal location of this image formation optical system, and at least one grating which divides into two or more wavelength the fluorescence which passed this confocal diaphragm, The photodetector which detects the light from said sample in which the spectrum was carried out by this grating, and the flying spot microscope characterized by providing at least one slit in which adjustable is possible for the width of face of the light introduced into this photodetector from said grating.
[Claim 2] Said laser light source means is a flying spot microscope according to claim 1 characterized by carrying out outgoing radiation of at least two or more waves of laser beams, and irradiating a sample.
[Claim 3] A laser light source means to scan the laser beam of single wavelength at least, and to irradiate a sample, The detection optical system which detects the light from said sample, and the image formation optical system which carries out image formation of the light from said sample, The confocal diaphragm arranged in the focal location of this image formation optical system, and the collimation optical system which makes a parallel ray emission light which passed this confocal diaphragm, The flying spot microscope characterized by providing at least one dichroic mirror which is arranged behind this collimation optical system and carries out the spectrum of the fluorescence from said sample with the predetermined spectral characteristic, and the photodetector which detects the light from the sample by which the spectrum was carried out with this dichroic mirror.

[Translation done.]

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the flying spot microscope possessing the fluorescence detection optical system and fluorescence observation optical system more than a single.
[0002]
[Description of the Prior Art]
Conventionally [<1st conventional example>], as a flying spot microscope in which fluorescence observation is possible, it is indicated by the U.S. Pat. No. 4997242 number specification, this performs a single or two fluorescence observation using the laser light source of single wavelength, and drawing 7 is drawing for explaining the 1st conventional example. As shown in drawing 7 , the laser beam injected from the laser oscillation machine 24 is scanned by the optical scan member 25 described below.
[0003] That is, it is reflected by the beam splitter 23, and after carrying out a two-dimensional scan by galvanometer 46b and plane mirror 35b which constitute galvanometer 46a which constitutes the 1st galvanometer scanner, plane mirror 35a, concave mirrors 42a and 42b, and a galvanometer scanner, it irradiates on a sample through a microscope. After the fluorescence emitted in the sample following a reverse optical path and passing a beam splitter 32 by this, it passes photomultiplier 30 at the time of single fluorescence, and the spectrum of the time of two fluorescence is carried out by the beam splitter 32, and it is respectively detected by a photomultiplier 30 and the photomultiplier 34. In addition, it has an ocular 27 and the diaphrams 31 and 33 for irises in addition to the configuration described above.
[0004] In the thing of such a configuration, after the fluorescence from the sample which is not illustrated reflects an ocular 27, plane mirror 35b, and concave mirrors 42a and 42b, it becomes a parallel ray, and is reflected by plane mirror 35a, and it passes the diaphrams 31 and 33 for irises, and is detected by photomultipliers 30 and 34.
[0005] The confocal effectiveness is acquired by the image formation optical system of the 1st conventional example described above taking the large optical path from diaphrams 31 and 33 to concave mirror 42a. Since the fluorescence from a sample becomes a parallel ray from concave mirror 42a before a photomultiplier 34 when an objective lens is a focal location, the light beam diameter led to a photomultiplier 34 is determined for the diameter of diaphrams 31 and 33.
[0006] As shown in <2nd conventional example> drawing 8 , the laser beam from a laser light source 1 The beam expander 2 which is the optical system for expanding to the beam diameter which becomes suitably A passage, After expanding a beam diameter, choose laser wavelength by the laser line filter 3 for choosing laser wavelength, and it is reflected with a dichroic mirror 4. XY polarization is carried out by the X-Y scan optical system 5, such as a galvanomirror, and through the pupil lens 6 and a microscope 7, a laser beam will be irradiated on a sample 19 and will carry out the beam scan of the sample 19.
[0007] The spectrum of the light which passed return and a dichroic mirror 4 is carried out with a dichroic mirror 64 in a path with the fluorescence from a microscope 7 to [path] a dichroic mirror 4 from the sample 19 excited by this, and one side passes along the image formation lens

71, and is detected by the photodetector 15 through the confocal diaphragm 74.
[0008] Similarly, it is reflected by the mirror 66, and the fluorescence which the spectrum of another side was carried out with the dichroic mirror 65, passed along the image formation lens 72, and was detected by the photodetector 14 through the confocal diaphragm 75, and passed the dichroic mirror 65 passes along the image formation lens 73, and is detected by the photodetector 13 through the confocal diaphragm 76.
[0009] The 2nd conventional example described above can lead the fluorescence from a sample 19 to the light-receiving field of a photodetector 15 by setting the distance l to a photodetector 15 to the focal distance f of the image formation lens 71, and the confocal diaphragm 74 suitably. Similarly, the fluorescence from a sample 19 can be led to the light-receiving field of photodetectors 14 and 13 by setting the distance l to photodetectors 14 and 13 to the focal distance f of the image formation lenses 72 and 73, and the confocal diaphragms 75 and 76 suitably.
[0010] <3rd conventional example> drawing 9 constitutes drawing 8 as follows. That is, the dichroic mirrors 64 and 65 of drawing 8 , the image formation lenses 71 and 72 currently arranged among photodetectors 15 and 14, respectively, the confocal diaphragms 74 and 75 and a mirror 66, the image formation lens 73 currently arranged between photodetectors 13, and the confocal diaphragm 76 are not established, but the image formation lens 77 and the confocal diaphragm 78 are established among dichroic mirrors 4 and 64.
[0011] Thus, if it is made a configuration like the 3rd conventional example, while the same function as drawing 8 will be obtained, compared with the conventional example of drawing 8 , equipment becomes easy and is that the cost of it is cut down.
The <4th conventional example> Conventionally, as a flying spot microscope in which fluorescence detection is possible, it is indicated by the U.S. Pat. No. 5127730 number specification, this detects two fluorescence using the laser light source of two or more wavelength, and drawing 10 is drawing for explaining the 4th conventional example again. As a laser light source 50, the Kr-Ar laser light source 50 which carries out the coincidence oscillation of the laser beam (488nm, 568nm, and 647nm) is used.
[0012] The laser beam 51 of three wavelength oscillated from the laser light source 50 is set to two, 488nm and 568nm, by dual band pass filter 52a of an excitation filter 52, and is led to the fluorescence sample 55 through the objective lens (not shown) of the lower part of drawing with the dual dichroic mirror 54. Said objective lens and the dual dichroic mirror 54 are penetrated, it is reflected with a reflecting mirror 56, and two kinds of wavelength which emitted light as fluorescence from the sample 55 is led to the filter block 57. And a spectrum is carried out to each wavelength by dichroic mirror 57a for a photometry which it has in the filter block 57, and it is detected by photomultipliers (PMT) 58 and 59 respectively through Filters 57b and 57c.
[0013] Thus, according to the flying spot microscope shown in drawing 10 , double excitation observation can be performed combining the multi-line laser light source which oscillates two or more wavelength.
The <5th conventional example> On the other hand, the flying spot microscope which detects three fluorescence for the 5th conventional example is indicated, and drawing 11 is drawing for explaining this. As for the laser beam (488nm and 514nm) 161 oscillated from the laser light source 160, only one one of wavelength is penetrated with the EKUSUTANARU filter 162.
[0014] And it is reflected in an illustration lower part by the beam splitter 163, passes along XY scanning unit 164, and is condensed by the sample 165 through the ocular 166 and objective lens 167 in an optical microscope. In this case, the 165th page of a sample is the scanning unit 164, and two-dimensional scans the 165th page of a sample. Then, the fluorescence which emitted light from the sample 165 passes along an objective lens 167, an ocular 166, and the scanning unit 164.
[0015] And a beam splitter 163 is penetrated, the spectrum of the case of a two-wave photometry is carried out by the beam splitter 168, one of these is led to a photomultiplier 174, the spectrum of the spectrum of another side of a beam splitter 168 is carried out by the beam splitter 169, one of these is detected by the photomultiplier 173 through a filter 172, and another side of the spectrum of a beam splitter 169 is detected by the photomultiplier 171 through a

filter 170.
[0016] Thus, suitably, the fluorescence from the irradiated laser beam 161 passes a beam splitter 163, and a spectrum is carried out by beam splitters 168 and 169, and it is respectively detected by the sample 165 by the photomultiplier 171,173,174.
[0017] In recent years, in fluorescence observation, not only a simple stain but the multiple stain is used abundantly. It carries out in order to visualize not only fluorescent staining but also a cell and the specific object of an in-house (singularity). Therefore, the indicator of the time of the multiple stain must be carried out as a difference in the difference of a color with each clear dyeing part, i.e., fluorescence wavelength.
[0018] By the way, if there are the various approaches in fluorescent staining extremely and the multiple stain is performed, a partial lap part (crossover part) may arise on fluorescence wavelength, and, as for drawing 12 , it is shown.
[0019]
[Problem(s) to be Solved by the Invention] In the 1st conventional example mentioned above, in order to acquire the confocal effectiveness, the large optical path length from concave mirrors 42a and 42b to diaphragms 31 and 33 must be taken, and equipment enlarges him. Although it is made to reflect like the mirrors 100a, 100b, and 100c of drawing 7 , and there is nothing if it is **** since the 1st conventional example is furthermore miniaturized, this becomes the cause which loses the quantity of light.
[0020] In the 2nd conventional example of drawing 8 , since the confocal diaphragms 74-76 and the image formation lenses 15, 14, and 13 are needed for each channel, equipment becomes complicated and serves as cost quantity. In the 3rd conventional example of drawing 9 , since dichroic mirrors 64 and 65 are arranged and it has three-channel composition, in a photodetector 14, the optical path length becomes large rather than 15, and, as for a photodetector 13, the optical path length becomes large rather than 14. Therefore, a light beam is no longer completely led to the light-receiving field of photodetectors 13 and 14.
[0021] In the 3rd conventional example of drawing 9 , in order to lead the fluorescence from a sample 19 to the light-receiving field of photodetectors 15, 14, and 13 without a loss, the focal distance f of the image formation lens 77 must be enlarged. However, when a focal distance f is enlarged, there is a fault that equipment is enlarged.
[0022] Moreover, as a means to divide two or more fluorescence wavelength depended on these multiple stain, beam splitters (dichroic mirror) 54 or 163 are used, and in order to limit the fluorescence wavelength detected further, it is necessary to use the sharp cut filter and band pass filters 52a, 52b, 52c, 57a, 57b, 57c, or 168,169,170,172 of various classes in the 4th conventional example of drawing 10 , or the 5th conventional example of drawing 11 . To compensate for the laser wavelength and fluorescent staining to be used, it is necessary to prepare these each time.
[0023] Generally, the filters 52a-52c with these wavelength dependencies, 57a-57c, and 168-170,172 are nonlinearity, and when it is going to remove the crossover of fluorescence wavelength, before they detect most part of the amount of fluorescence, they will be left. S/N of detection falls by this. Moreover, depending on the class of dyeing, what has the large crossover of fluorescence wavelength exists. Therefore, in the 4th conventional example or the 5th conventional example mentioned above, this detection itself becomes impossible.
[0024] It is in the 1st purpose of this invention offering the flying spot microscope which can perform good detection of S/N, without using filters with the wavelength dependency for canceling said fault and detecting each fluorescence wavelength at the time of the multiple stain.

[0025] It is in the 2nd purpose of this invention offering the flying spot microscope which can cancel said fault, can lead without a loss the fluorescence acquired by reflecting a sample to a photodetector, and moreover becomes small and cheap.
[0026]
[Means for Solving the Problem] In order to attain said purpose, invention corresponding to claim 1 A laser light source means to scan the laser beam of single wavelength at least, and to irradiate a sample, The detection optical system which detects the light from said sample, and

the image formation optical system which carries out image formation of the light from said sample. The confocal diaphragm arranged in the focal location of this image formation optical system, and at least one grating which divides into two or more wavelength the fluorescence which passed this confocal diaphragm, They are the photodetector which detects the light from said sample in which the spectrum was carried out by this grating, and the flying spot microscope characterized by providing at least one slit in which adjustable is possible for the width of face of the light introduced into this photodetector from said grating.

[0027] In order to attain said purpose, invention corresponding to claim 2 is a flying spot microscope according to claim 1 characterized by carrying out outgoing radiation of at least two or more waves of laser beams as said laser light source means, and irradiating a sample.

[0028] In order to attain said purpose, invention corresponding to claim 3 A laser light source means to scan the laser beam of single wavelength at least, and to irradiate a sample,

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TECHNICAL FIELD

[Industrial Application] This invention relates to the flying spot microscope possessing the fluorescence detection optical system and fluorescence observation optical system more than a single.

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PRIOR ART

[Description of the Prior Art]
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[0003] That is, it is reflected by the beam splitter 23, and after carrying out a two-dimensional scan by galvanometer 46b and plane mirror 35b which constitute galvanometer 46a which constitutes the 1st galvanometer scanner, plane mirror 35a, concave mirrors 42a and 42b, and a galvanometer scanner, it irradiates on a sample through a microscope. After the fluorescence emitted in the sample following a reverse optical path and passing a beam splitter 32 by this, it passes photomultiplier 30 at the time of single fluorescence, and the spectrum of the time of two fluorescence is carried out by the beam splitter 32, and it is respectively detected by a photomultiplier 30 and the photomultiplier 34. In addition, it has an ocular 27 and the diaphrams 31 and 33 for irises in addition to the configuration described above.
[0004] In the thing of such a configuration, after the fluorescence from the sample which is not illustrated reflects an ocular 27, plane mirror 35b, and concave mirrors 42a and 42b, it becomes a parallel ray, and is reflected by plane mirror 35a, and it passes the diaphrams 31 and 33 for irises, and is detected by photomultipliers 30 and 34.
[0005] The confocal effectiveness is acquired by the image formation optical system of the 1st conventional example described above taking the large optical path from diaphrams 31 and 33 to concave mirror 42a. Since the fluorescence from a sample becomes a parallel ray from concave mirror 42a before a photomultiplier 34 when an objective lens is a focal location, the light beam diameter led to a photomultiplier 34 is determined for the diameter of diaphrams 31 and 33.
[0006] As shown in <2nd conventional example> drawing 8 , the laser beam from a laser light source 1 The beam expander 2 which is the optical system for expanding to the beam diameter which becomes suitably A passage, After expanding a beam diameter, choose laser wavelength by the laser line filter 3 for choosing laser wavelength, and it is reflected with a dichroic mirror 4. XY polarization is carried out by the X-Y scan optical system 5, such as a galvanomirror, and through the pupil lens 6 and a microscope 7, a laser beam will be irradiated on a sample 19 and will carry out the beam scan of the sample 19.
[0007] The spectrum of the light which passed return and a dichroic mirror 4 is carried out with a dichroic mirror 64 in a path with the fluorescence from a microscope 7 to [path] a dichroic mirror 4 from the sample 19 excited by this, and one side passes along the image formation lens 71, and is detected by the photodetector 15 through the confocal diaphragm 74.
[0008] Similarly, it is reflected by the mirror 66, and the fluorescence which the spectrum of another side was carried out with the dichroic mirror 65, passed along the image formation lens 72, and was detected by the photodetector 14 through the confocal diaphragm 75, and passed the dichroic mirror 65 passes along the image formation lens 73, and is detected by the photodetector 13 through the confocal diaphragm 76.

[0009] The 2nd conventional example described above can lead the fluorescence from a sample 19 to the light-receiving field of a photodetector 15 by setting the distance l to a photodetector 15 to the focal distance f of the image formation lens 71, and the confocal diaphragm 74 suitably. Similarly, the fluorescence from a sample 19 can be led to the light-receiving field of photodetectors 14 and 13 by setting the distance l to photodetectors 14 and 13 to the focal distance f of the image formation lenses 72 and 73, and the confocal diaphragms 75 and 76 suitably.
[0010] <3rd conventional example> drawing 9 constitutes drawing 8 as follows. That is, the dichroic mirrors 64 and 65 of drawing 8 , the image formation lenses 71 and 72 currently arranged among photodetectors 15 and 14, respectively, the confocal diaphragms 74 and 75 and a mirror 66, the image formation lens 73 currently arranged between photodetectors 13, and the confocal diaphragm 76 are not established, but the image formation lens 77 and the confocal diaphragm 78 are established among dichroic mirrors 4 and 64.
[0011] Thus, if it is made a configuration like the 3rd conventional example, while the same function as drawing 8 will be obtained, compared with the conventional example of drawing 8 , equipment becomes easy and is that the cost of it is cut down.
The <4th conventional example> Conventionally, as a flying spot microscope in which fluorescence detection is possible, it is indicated by the U.S. Pat. No. 5127730 number specification, this detects two fluorescence using the laser light source of two or more wavelength, and drawing 10 is drawing for explaining the 4th conventional example again. As a laser light source 50, the Kr-Ar laser light source 50 which carries out the coincidence oscillation of the laser beam (488nm, 568nm, and 647nm) is used.
[0012] The laser beam 51 of three wavelength oscillated from the laser light source 50 is set to two, 488nm and 568nm, by dual band pass filter 52a of an excitation filter 52, and is led to the fluorescence sample 55 through the objective lens (not shown) of the lower part of drawing with the dual dichroic mirror 54. Said objective lens and the dual dichroic mirror 54 are penetrated, it is reflected with a reflecting mirror 56, and two kinds of wavelength which emitted light as fluorescence from the sample 55 is led to the filter block 57. And a spectrum is carried out to each wavelength by dichroic mirror 57a for a photometry which it has in the filter block 57, and it is detected by photomultipliers (PMT) 58 and 59 respectively through Filters 57b and 57c.
[0013] Thus, according to the flying spot microscope shown in drawing 10 , double excitation observation can be performed combining the multi-line laser light source which oscillates two or more wavelength.
The <5th conventional example> On the other hand, the flying spot microscope which detects three fluorescence for the 5th conventional example is indicated, and drawing 11 is drawing for explaining this. As for the laser beam (488nm and 514nm) 161 oscillated from the laser light source 160, only one one of wavelength is penetrated with the EKUSUTANARU filter 162.
[0014] And it is reflected in an illustration lower part by the beam splitter 163, passes along XY scanning unit 164, and is condensed by the sample 165 through the ocular 166 and objective lens 167 in an optical microscope. In this case, the 165th page of a sample is the scanning unit 164, and two-dimensional scans the 165th page of a sample. Then, the fluorescence which emitted light from the sample 165 passes along an objective lens 167, an ocular 166, and the scanning unit 164.
[0015] And a beam splitter 163 is penetrated, the spectrum of the case of a two-wave photometry is carried out by the beam splitter 168, one of these is led to a photomultiplier 174, the spectrum of the spectrum of another side of a beam splitter 168 is carried out by the beam splitter 169, one of these is detected by the photomultiplier 173 through a filter 172, and another side of the spectrum of a beam splitter 169 is detected by the photomultiplier 171 through a filter 170.
[0016] Thus, suitably, the fluorescence from the irradiated laser beam 161 passes a beam splitter 163, and a spectrum is carried out by beam splitters 168 and 169, and it is respectively detected by the sample 165 by the photomultiplier 171,173,174.
[0017] In recent years, in fluorescence observation, not only a simple stain but the multiple stain is used abundantly. It carries out in order to visualize not only fluorescent staining but also a cell

and the specific object of an in-house (singularity). Therefore, the indicator of the time of the multiple stain must be carried out as a difference in the difference of a color with each clear dyeing part, i.e., fluorescence wavelength.

[0018] By the way, if there are the various approaches in fluorescent staining extremely and the multiple stain is performed, a partial lap part (crossover part) may arise on fluorescence wavelength, and, as for drawing 12 , it is shown.

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EFFECT OF THE INVENTION

[Effect of the Invention] The possible flying spot microscope of good fluorescence detection of S/N without the fluorescence crossover at the time of the multiple stain can be offered without using the filters which have a wavelength dependency as a photometry separation means according to this invention. Moreover, according to this invention, the fluorescence acquired by reflecting a sample can be led to a photodetector without a loss, and the flying spot microscope which moreover becomes small and cheap can be offered.

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] In the 1st conventional example mentioned above, in order to acquire the confocal effectiveness, the large optical path length from concave mirrors 42a and 42b to diaphragms 31 and 33 must be taken, and equipment enlarges him. Although it is made to reflect like the mirrors 100a, 100b, and 100c of drawing 7 , and there is nothing if it is **** since the 1st conventional example is furthermore miniaturized, this becomes the cause which loses the quantity of light.
[0020] In the 2nd conventional example of drawing 8 , since the confocal diaphragms 74-76 and the image formation lenses 15, 14, and 13 are needed for each channel, equipment becomes complicated and serves as cost quantity. In the 3rd conventional example of drawing 9 , since dichroic mirrors 64 and 65 are arranged and it has three-channel composition, in a photodetector 14, the optical path length becomes large rather than 15, and, as for a photodetector 13, the optical path length becomes large rather than 14. Therefore, a light beam is no longer completely led to the light-receiving field of photodetectors 13 and 14.
[0021] In the 3rd conventional example of drawing 9 , in order to lead the fluorescence from a sample 19 to the light-receiving field of photodetectors 15, 14, and 13 without a loss, the focal distance f of the image formation lens 77 must be enlarged. However, when a focal distance f is enlarged, there is a fault that equipment is enlarged.
[0022] Moreover, as a means to divide two or more fluorescence wavelength depended on these multiple stain, beam splitters (dichroic mirror) 54 or 163 are used, and in order to limit the fluorescence wavelength detected further, it is necessary to use the sharp cut filter and band pass filters 52a, 52b, 52c, 57a, 57b, 57c, or 168,169,170,172 of various classes in the 4th conventional example of drawing 10 , or the 5th conventional example of drawing 11 . To compensate for the laser wavelength and fluorescent staining to be used, it is necessary to prepare these each time.
[0023] Generally, the filters 52a-52c with these wavelength dependencies, 57a-57c, and 168-170,172 are nonlinearity, and when it is going to remove the crossover of fluorescence wavelength, before they detect most part of the amount of fluorescence, they will be left. S/N of detection falls by this. Moreover, depending on the class of dyeing, what has the large crossover of fluorescence wavelength exists. Therefore, in the 4th conventional example or the 5th conventional example mentioned above, this detection itself becomes impossible.
[0024] It is in the 1st purpose of this invention offering the flying spot microscope which can perform good detection of S/N, without using filters with the wavelength dependency for canceling said fault and detecting each fluorescence wavelength at the time of the multiple stain.

[0025] It is in the 2nd purpose of this invention offering the flying spot microscope which can cancel said fault, can lead without a loss the fluorescence acquired by reflecting a sample to a photodetector, and moreover becomes small and cheap.

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MEANS

[Means for Solving the Problem] In order to attain said purpose, invention corresponding to claim 1 A laser light source means to scan the laser beam of single wavelength at least, and to irradiate a sample, The detection optical system which detects the light from said sample, and the image formation optical system which carries out image formation of the light from said sample, The confocal diaphragm arranged in the focal location of this image formation optical system, and at least one grating which divides into two or more wavelength the fluorescence which passed this confocal diaphragm, They are the photodetector which detects the light from said sample in which the spectrum was carried out by this grating, and the flying spot microscope characterized by providing at least one slit in which adjustable is possible for the width of face of the light introduced into this photodetector from said grating.
[0027] In order to attain said purpose, invention corresponding to claim 2 is a flying spot microscope according to claim 1 characterized by carrying out outgoing radiation of at least two or more waves of laser beams as said laser light source means, and irradiating a sample.
[0028] In order to attain said purpose, invention corresponding to claim 3 A laser light source means to scan the laser beam of single wavelength at least, and to irradiate a sample, The detection optical system which detects the light from said sample, and the image formation optical system which carries out image formation of the light from said sample, The confocal diaphragm arranged in the focal location of this image formation optical system, and the collimation optical system which makes a parallel ray emission light which passed this confocal diaphragm, It is the flying spot microscope characterized by providing the photodetector which detects the light from the sample by which the spectrum was carried out with at least one dichroic mirror which is arranged behind this collimation optical system and carries out the spectrum of the fluorescence from said sample with the predetermined spectral characteristic, and this dichroic mirror.
[0029]

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OPERATION

[Function] Since according to invention corresponding to claim 1 the laser beam of single wavelength is irradiated at a sample, and the fluorescence to which it comes from a sample is separated by the photometry separation means and it is detected by the photodetector, S/N is good and separation of fluorescence wavelength can be performed.
[0030] Since according to invention corresponding to claim 2 two or more waves of laser beams are irradiated at a sample, the fluorescence to which it comes from a sample is separated by the photometry separation means and it is detected by the photodetector, separation of still more various fluorescence wavelength can be performed compared with invention corresponding to claim 1.
[0031] According to invention corresponding to claim 3, the fluorescence acquired by reflecting a sample with at least one dichroic mirror can be led to a photodetector without a loss, and, moreover, it becomes small and cheap.

[Translation done.]

EXAMPLE

[Example] Hereafter, the example of this invention is explained with reference to a drawing.
<1st example> drawing 1 is drawing showing the optical system of the 1st example of the flying spot microscope of this invention. A laser light source 1 carries out outgoing radiation of single wavelength, for example, the 488nm laser beam, and this example irradiates a sample 19. The laser beam from a laser light source 1 is led to the laser light source means and the photometry separation means of mentioning later. The laser light source means is constituted so that it may lead to a sample 19 through a beam expander 2, a dichroic mirror 4, the X-Y scan optical system 5, the pupil projection lens 6, and a microscope 7 one by one.
[0033] Moreover, a photometry separation means consists of the slits 10, 11, and 12 which can change the confocal optical system 8, a grating 9, and width of face, condenser lenses 16, 17, and 18, and photodetectors 13, 14, and 15, after separating the fluorescence from a sample 19 with a dichroic mirror 4.
[0034] In the thing of such a configuration, the fluorescence emitted from the sample 19 results in a grating 9, after passing the confocal optical system 8 from a microscope 7. Of course, it is possible at this time to also make the confocal optical system 8 bypass. The fluorescence which resulted in the grating 9 is doubled with the wavelength, and is divided into zero-order - the n-th light. Slits 10, 11, and 12, condenser lenses 16, 17, and 18, and photodetectors 13, 14, and 15 correspond to each of these **** respectively. Respectively, modification of each fluorescence wavelength range to detect is attained by changing the width of face of slits 10, 11, and 12.
[0035] Since the filters which have wavelength dependencies, such as a dichroic mirror, a sharp cut filter, and a PANDO pass filter, as a photometry separation means are not used according to the 1st example described above, good detection of S/N without the fluorescence crossover at the time of the multiple stain is attained.
[0036] the configuration which <2nd example> drawing 2 is drawing showing the optical system of the 2nd example of this invention, and newly forms a dichroic mirror 22 and the laser line filter 3 on the optical path between the beam expander 2 of the 1st above-mentioned example, and a dichroic mirror 4, expands the laser beam from a single or two or more wave coincidence oscillation laser light source 20 to a beam expander 2, and irradiates a dichroic mirror 22 further -- it is constituted like. After being reflected with dichroic mirrors 4 other than this, the configuration until it results in detectors 13, 14, and 15 is the same as that of the 1st example.
[0037] As a laser light source 20, what combined the Ar-Kr laser light source (488nm and 568nm) and 351nm Ar laser light source is used. The operation effectiveness as the 1st above-mentioned example also with the 2nd same example described above is acquired. That is, since two or more waves of laser beams from laser light sources 1 and 20 are irradiated at a sample 19, the fluorescence to which it comes from a sample 19 is separated by the grating 9 and it is detected by photodetectors 13, 14, and 15, S/N is good and separation of still more various fluorescence wavelength can be performed compared with the 1st example.
[0038] It is drawing showing the optical system of the 3rd example of this invention, and <3rd example> drawing 3 changes the laser light source 1 of the 1st above-mentioned example into two or more wave coincidence oscillation laser light source 21 which consists of a multi-line Ar laser light source (351nm, 458nm, 488nm, and 514.5nm), forms the laser line filter 3 between a

beam expander 2 and a dichroic mirror 4, and that of the configuration of those other than this is the same as that of the 1st above-mentioned example.
[0039] Since according to the 3rd example two or more waves of laser beams from a laser light source 21 are irradiated at a sample 19, the fluorescence to which it comes from a sample 19 is separated by the grating 9 and it is detected by photodetectors 13, 14, and 15, S/N is good and separation of still more various fluorescence wavelength can be performed compared with the 1st example.
[0040] <4th example> drawing 4 is drawing showing the optical system of the 4th example of this invention, and a different point from the example of drawing 1 is constituted as follows. Namely, the image formation lens 61 which condenses the reflected light from a sample 19 and the confocal diaphragm 62 arranged in the image formation location of the image formation lens 61, The collimation optical system 63 which makes a parallel ray emission light (beam with a flare angle) which passes this confocal diaphragm 62, Two dichroic mirrors 64 and 65 which are arranged behind this collimation optical system 63, and carry out the spectrum of the fluorescence from a sample 19 with the predetermined spectral characteristic, It is arranged behind a dichroic mirror 65 and the mirror 66 which reflects the spectrum obtained from a dichroic mirror 65, and is led to a photodetector 13 is formed.
[0041] In the thing of such a configuration, the light (diffused light) which passed the confocal diaphragm 62 according to the collimation optical system 63 is changed into parallel light. Therefore, the strength of the light can be measured by each photodetectors 15 and 14 and 13 in the light of two or more wavelength which carries out the spectrum of the light after passing the confocal diaphragm 62 for every predetermined wavelength, and is different.
[0042] In this case, no matter it may arrange photodetectors 15, 14, and 13 in what distance from the confocal diaphragm 62, since a measuring beam bundle is changed into parallel light according to the collimation optical system 63, the total quantity of light does not have a loss and carries out incidence of it to photodetectors 15 and 14 and 13 through dichroic mirrors 64 and 65 and a mirror 66. Therefore, dichroic mirrors 64 and 65, photodetectors 15 and 14, and 13 can be arranged freely, without receiving the constraint on optics.
[0043] <5th example> drawing 5 is drawing showing the optical system of the 4th example of this invention, and makes collimation optical system 63 of drawing 4 the following collimation optical system 67. That is, one side uses as a plane convex lens, and one side uses collimation optical system 67 as the convex lens on the spherical surface, and it forms a pinhole in the vacuum evaporationo film which is not illustrated to the field by the side of the flat surface of the convex lens of a parenthesis.
[0044] Thus, by constituting, the light beam which passed the collimation optical system 67 becomes a parallel ray, a spectrum is carried out with dichroic mirrors 64 and 65, and it is drawn, without separating from a light-receiving field in photodetectors 15 and 14 and 13.
[0045] Since the collimation optical system 67 serves as the confocal diaphragm, it is made cheaply small.
It is drawing showing the optical system of the 6th example of this invention, and convex lens 67a and concave lens 67b should combine <6th example> drawing 6 (a), and it constitutes a photometry separation means from a grating 9, and constitutes the collimation optical system 67 of the example of drawing 5 from slits 10, 11, and 12 which can further change the width of face of light, condenser lenses 16, 17, and 18, and photodetectors 13, 14, and 15.
[0046] In the thing of such a configuration, the fluorescence emitted from the sample which is not illustrated passes the microscope which is not illustrated, a pupil projection lens, X-Y scan optical system, and a dichroic mirror, and results in a grating 9 through the joint lens 61, the confocal diaphragm 62, and the collimation optical system 67. Fluorescence is doubled with the wavelength and divided into zero-order - the n-th light. Slits 10-12, condenser lenses 16-18, and photodetectors 13-15 correspond to each of these dimensions respectively. Respectively, modification of each fluorescence wavelength range to detect is attained by changing the width of face of slits 10-12.
[0047] In the 6th example, collimation optical system 67 is made as for a miniaturization to making it the target beam diameter, when convex lens 67a and concave lens 67b should combine.

this comes out compared with the case where it constitutes only from a convex lens as collimation optical system 67, as shown in drawing 6 (b). In addition, it is necessary to enlarge a beam diameter enough to the lattice spacing of a grating 9.

[Translation done.]

* NOTICES *

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing showing the 1st example of the flying spot microscope of this invention.
[Drawing 2] Drawing showing the 2nd example of the flying spot microscope of this invention.
[Drawing 3] Drawing showing the 3rd example of the flying spot microscope of this invention.
[Drawing 4] Drawing showing the 4th example of the flying spot microscope of this invention.
[Drawing 5] Drawing showing the 5th example of the flying spot microscope of this invention.
[Drawing 6] Drawing showing the 6th example of the flying spot microscope of this invention.
[Drawing 7] Drawing showing the 1st conventional example.
[Drawing 8] Drawing showing the 2nd conventional example.
[Drawing 9] Drawing showing the 3rd conventional example.
[Drawing 10] Drawing showing the 4th conventional example.
[Drawing 11] Drawing showing the 5th conventional example.
[Drawing 12] Drawing showing the crossover of fluorescence.
[Description of Notations]
1 -- A single wavelength oscillation laser light source, 2 -- A beam expander, 4 -- Dichroic mirror, 5 [-- Confocal optical system,] -- XY scan optical system, 6 -- A projection-on pupil lens, 7 -- A microscope, 8 9 -- A grating, 10, 11, 12 -- An adjustable slit, 13, 14, 15 -- Photodetector, 16, 17, 18 [-- An image formation lens 62 / -- 63 A confocal diaphragm, 67 / -- 64 Collimator optical system 65 / -- A dichroic mirror, 66 / -- Mirror.] -- A condenser lens, 19 -- 21 A sample, 29 -- A laser light source, 61

[Translation done.]

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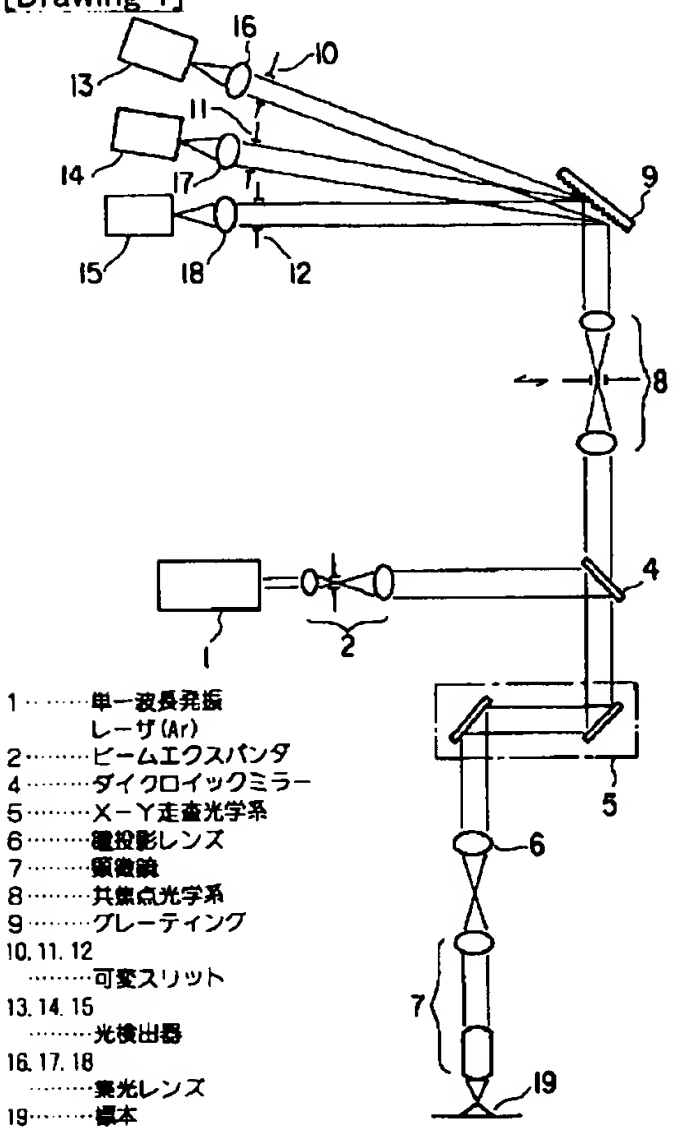
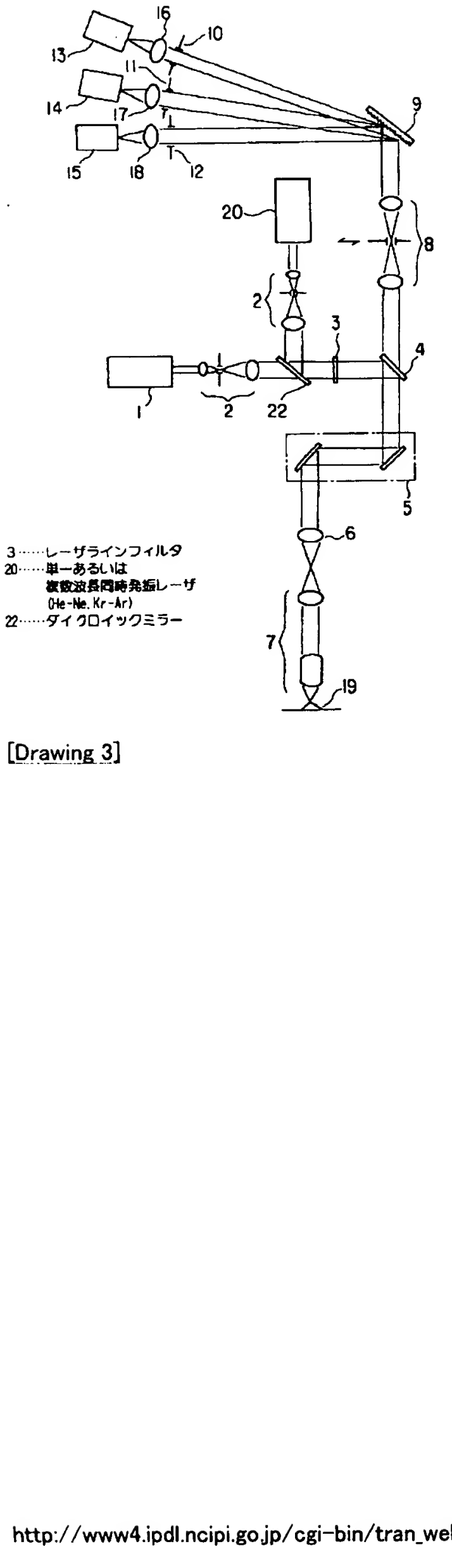
DRAWINGS

[Drawing 1]



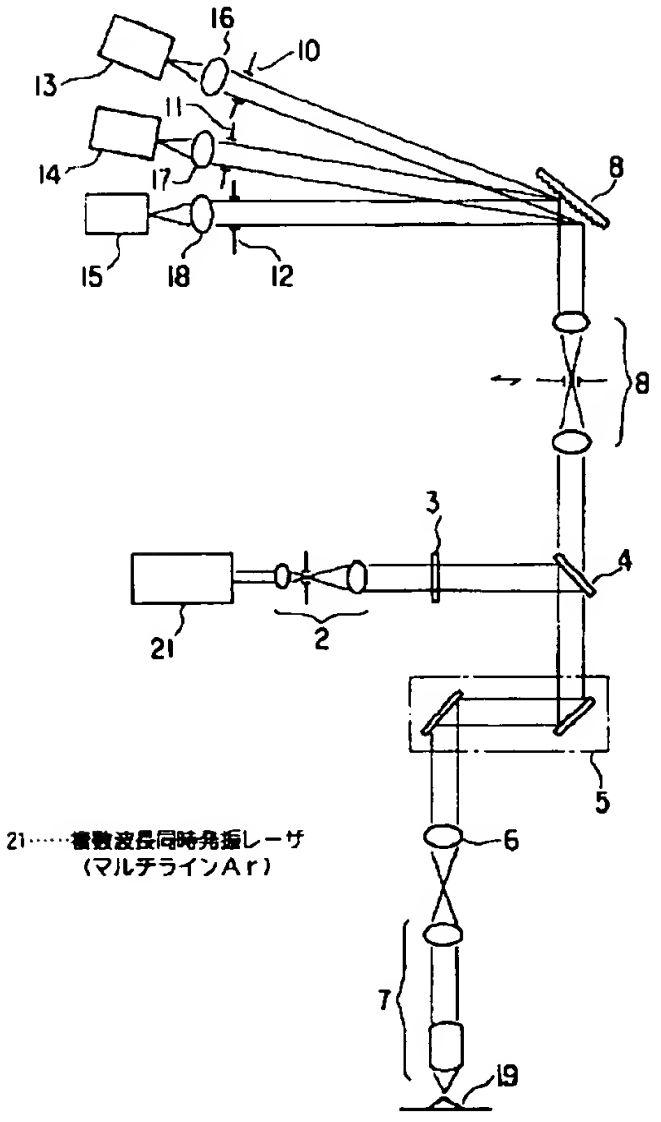
1……単一波長発振
レーザ(Ar)
2……ビームエクスパンダ
4……ダイクロイックミラー
5……X－Y走査光学系
6……螢投影レンズ
7……顕微鏡
8……共焦点光学系
9……グレーティング
10. 11. 12
……可変スリット
13. 14. 15
……光検出器
16. 17. 18
……集光レンズ
19……標本

[Drawing 2]

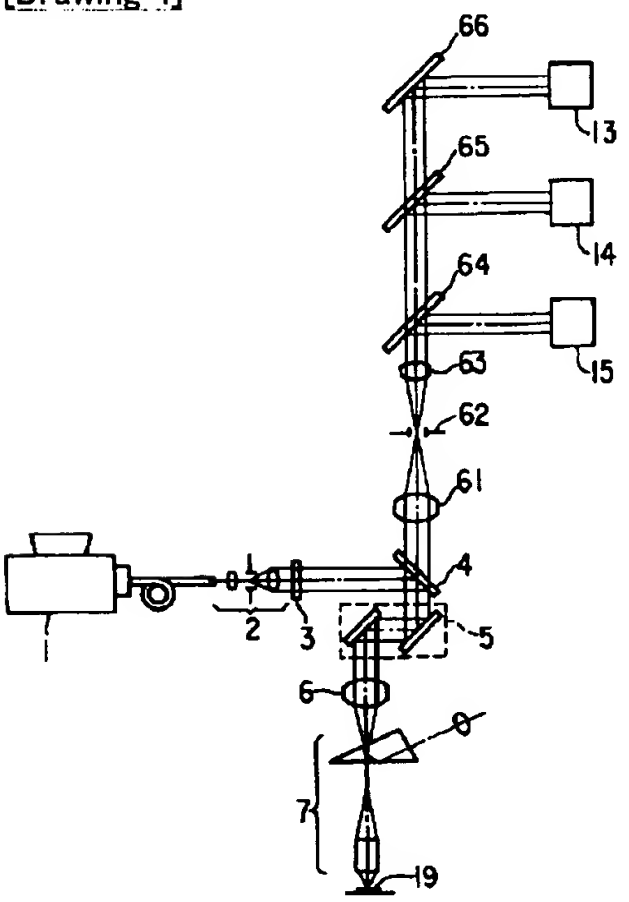


3……レーザラインフィルタ
20……単一あるいは
複数波長同時発振レーザ
(He-Ne, Kr-Ar)
22……ダイクロイックミラー

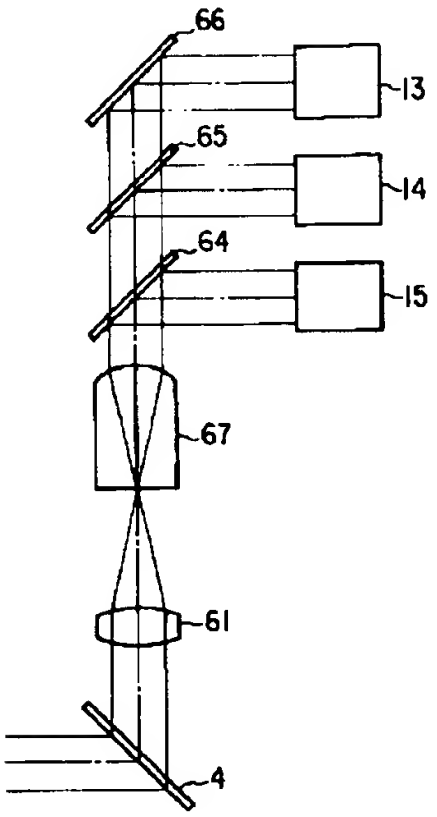
[Drawing 3]



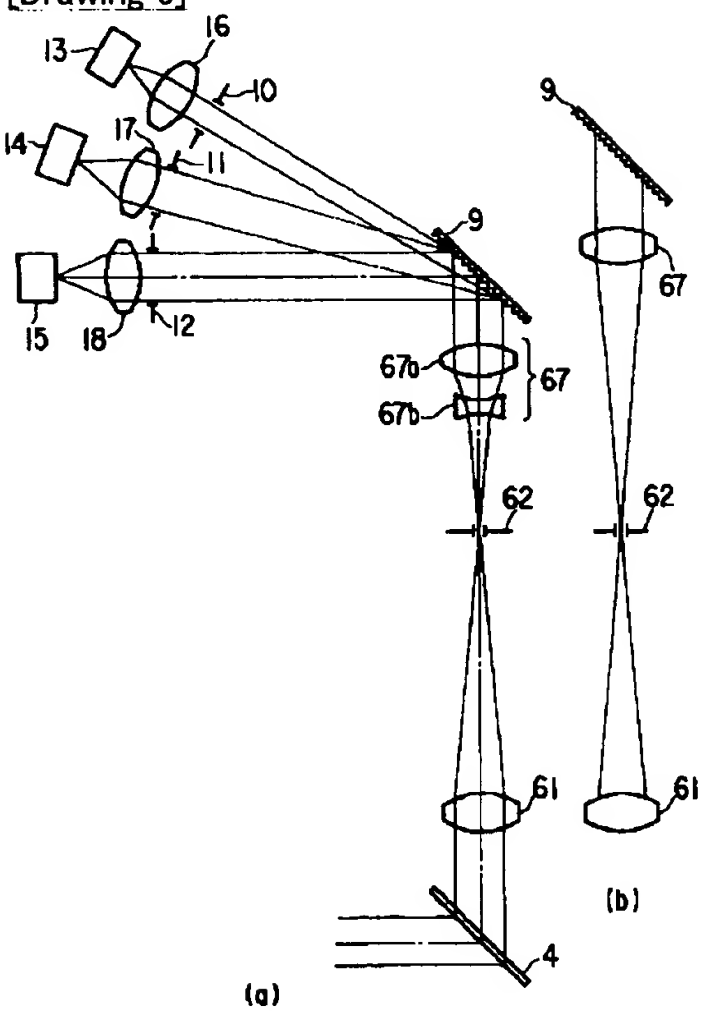
[Drawing 4]



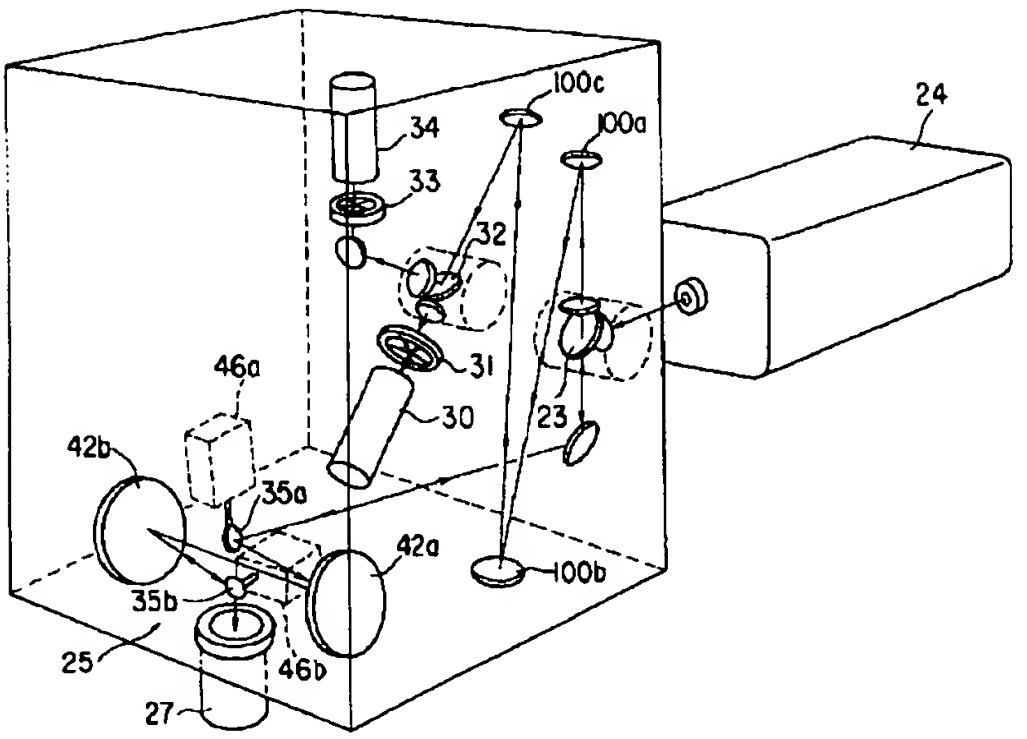
[Drawing 5]



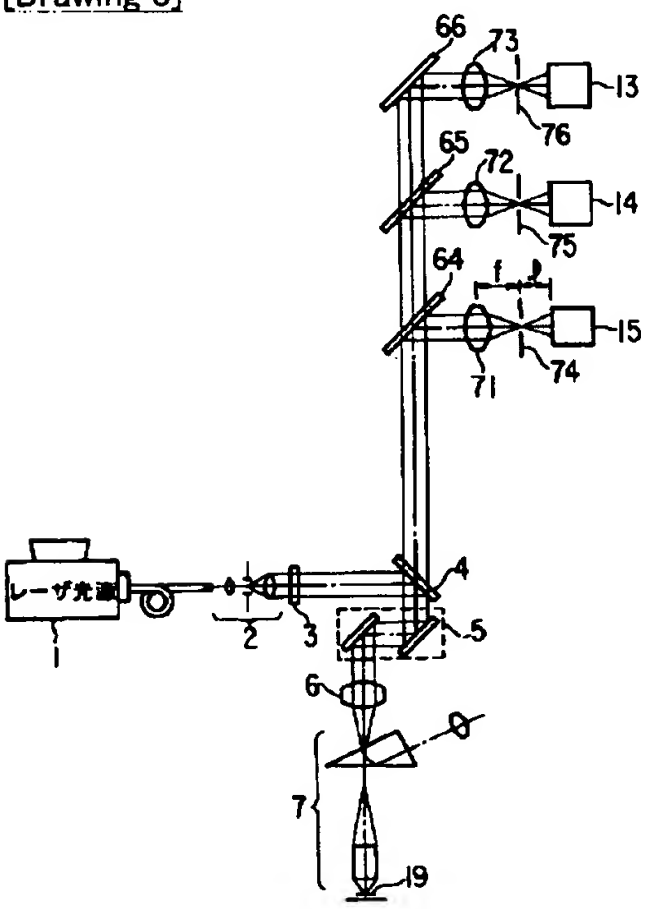
[Drawing 6]



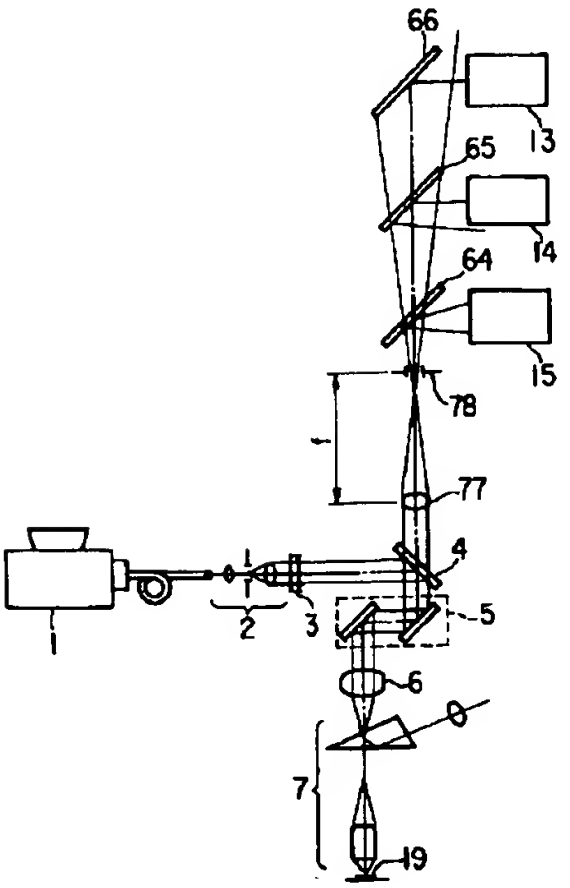
[Drawing 7]



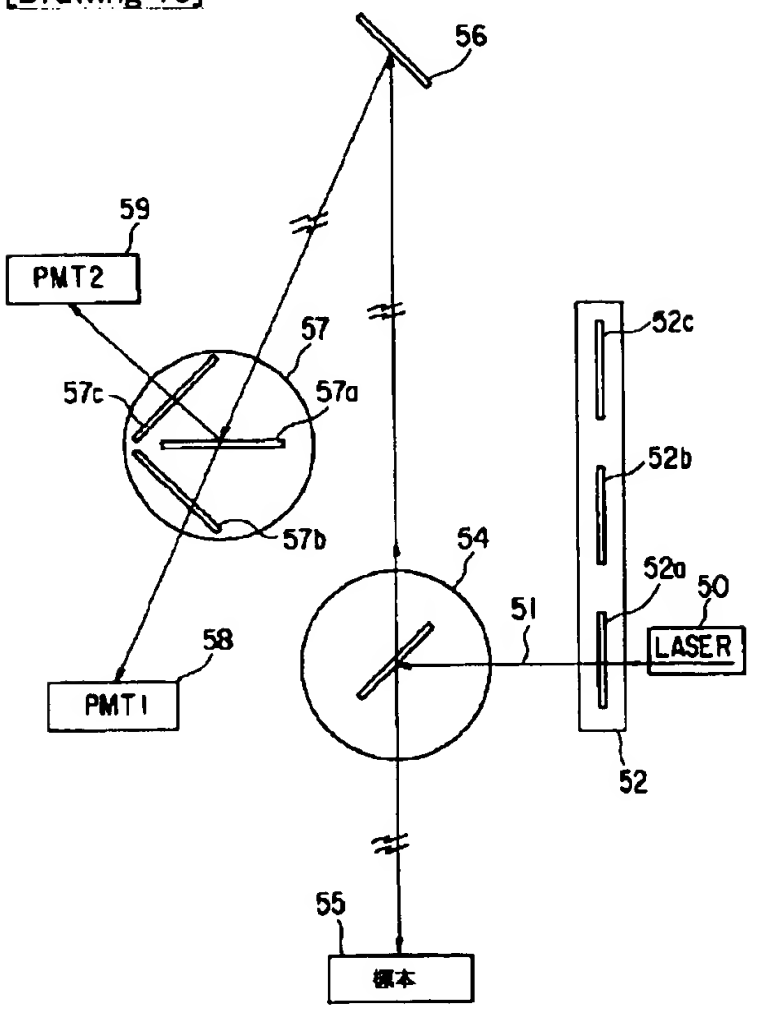
[Drawing 8]



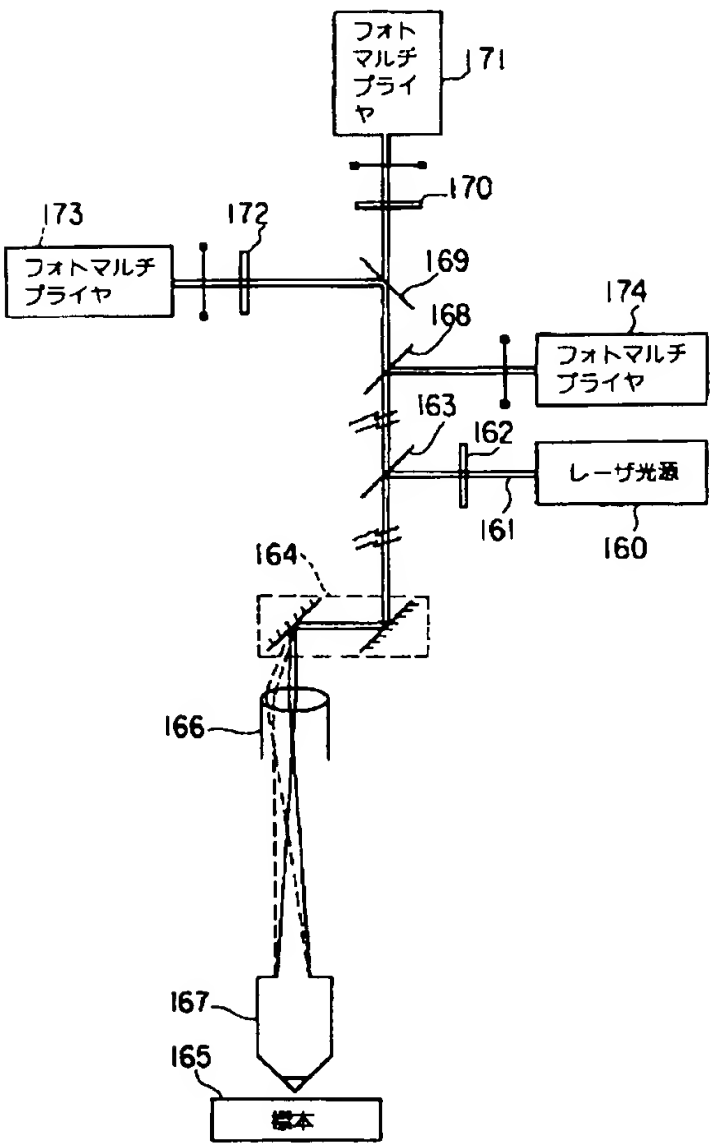
[Drawing 9]



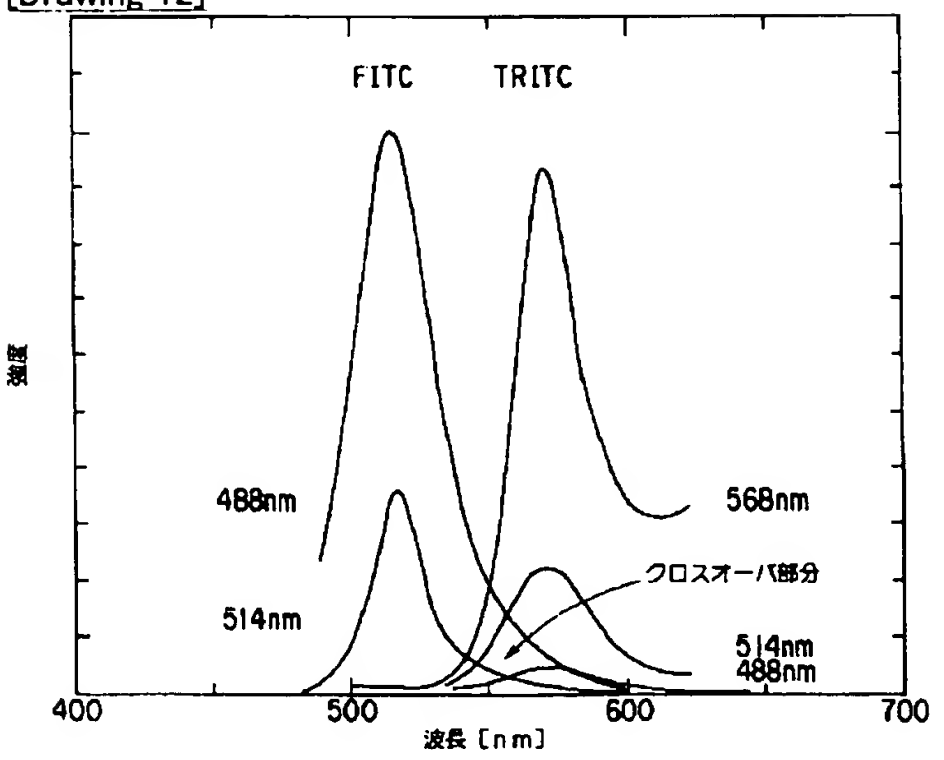
[Drawing 10]



[Drawing 11]



[Drawing 12]



[Translation done.]

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CORRECTION OR AMENDMENT

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[Filing Date] March 29, Heisei 12 (2000. 3.29)
[Procedure amendment 1]
[Document to be Amended] Specification
[Item(s) to be Amended] Claim
[Method of Amendment] Modification
[Proposed Amendment]
[Claim(s)]
[Claim 1] The laser light source which injects the laser beam of single wavelength at least,
A scan means to scan the laser beam from said laser light source to a sample,
Separation optical system which is arranged between said said laser light sources and scan means, and separates the laser beam from said laser light source, and the light from said sample,

The confocal diaphragm arranged in the location which the light from said sample separated by said separation optical system condenses,
At least one grating which divides into two or more wavelength the light from a sample which passed said confocal diaphragm,
The flying spot microscope characterized by providing the photodetector which detects the light from said sample in which the spectrum was carried out by said grating.
[Claim 2] Said laser light source,
The flying spot microscope according to claim 1 characterized by carrying out outgoing radiation of at least two or more waves of laser beams.
[Claim 3] Said flying spot microscope,

Furthermore, the flying spot microscope according to claim 1 or 2 characterized by having the parallel light conversion optical system which makes parallel light light which passed said confocal diaphragm.
[Claim 4] The laser light source which injects the laser beam of single wavelength at least,
A scan means to scan the laser beam from said laser light source to a sample,
Separation optical system which is arranged between said said laser light sources and scan means, and separates the laser beam from said laser light source, and the light from said sample,

The confocal diaphragm arranged in the location which the light from said sample separated by said separation optical system condenses,
Parallel light conversion optical system which makes parallel light light which passed said confocal diaphragm,
at least one spectrum which carries out the spectrum of the light from said sample which has been arranged behind said parallel light conversion optical system, passed said parallel light conversion optical system, and became parallel light to two or more wavelength -- optical system,
said spectrum -- the flying spot microscope characterized by providing the photodetector which detects the light from said sample by which the spectrum was carried out by optical system.
[Claim 5] said spectrum -- optical system,
The flying spot microscope according to claim 4 characterized by being the dichroic mirror which carries out the spectrum of the light from said sample which passed said parallel light conversion optical system, and became parallel light with the predetermined spectral characteristic.
[Procedure amendment 2]
[Document to be Amended] Specification
[Item(s) to be Amended] 0020
[Method of Amendment] Modification
[Proposed Amendment]
[0020] In the 2nd conventional example of drawing 8, since the confocal diaphragms 74-76 and the image formation lenses 71, 72, and 73 are needed for each channel, equipment becomes complicated and serves as cost quantity. In the 3rd conventional example of drawing 9, since dichroic mirrors 64 and 65 are arranged and it has three-channel composition, in a photodetector 14, the optical path length becomes large rather than 15, and, as for a photodetector 13, the optical path length becomes large rather than 14. Therefore, a light beam is no longer completely led to the light-receiving field of photodetectors 13 and 14.
[Procedure amendment 3]
[Document to be Amended] Specification
[Item(s) to be Amended] 0026
[Method of Amendment] Modification
[Proposed Amendment]
[0026]
[Means for Solving the Problem] In order to attain said purpose, invention corresponding to claim 1 The laser light source which injects the laser beam of single wavelength at least, and a scan means to scan the laser beam from said laser light source to a sample, The separation optical system which is arranged between said said laser light sources and scan means, and separates the laser beam from said laser light source, and the light from said sample, The confocal diaphragm arranged in the location which the light from said sample separated by said separation optical system condenses, It is the flying spot microscope characterized by providing at least one grating which divides into two or more wavelength the light from a sample which passed said confocal diaphragm, and the photodetector which detects the light from said sample in which the spectrum was carried out by said grating.
[Procedure amendment 4]
[Document to be Amended] Specification
[Item(s) to be Amended] 0027
[Method of Amendment] Modification

[Proposed Amendment]
[0027] In order to attain said purpose, invention corresponding to claim 2 is a flying spot microscope according to claim 1 characterized by carrying out outgoing radiation of at least two or more waves of laser beams as said laser light source means, and irradiating a sample. Moreover, invention corresponding to claim 3 is a flying spot microscope according to claim 1 or 2 characterized by having the parallel light conversion optical system which makes parallel light further light which passed said confocal diaphragm.
[Procedure amendment 5]
[Document to be Amended] Specification
[Item(s) to be Amended] 0028
[Method of Amendment] Modification
[Proposed Amendment]
[0028] In order to attain said purpose, invention corresponding to claim 4 The laser light source which injects the laser beam of single wavelength at least, and a scan means to scan the laser beam from said laser light source to a sample, The separation optical system which is arranged between said said laser light sources and scan means, and separates the laser beam from said laser light source, and the light from said sample, The confocal diaphragm arranged in the location which the light from said sample separated by said separation optical system condenses, It is arranged behind the parallel light conversion optical system which makes parallel light light which passed said confocal diaphragm, and said parallel light conversion optical system. at least one spectrum which carries out the spectrum of the light from said sample which passed said parallel light conversion optical system, and became parallel light to two or more wavelength -- optical system and said spectrum -- it is the flying spot microscope characterized by providing the photodetector which detects the light from said sample by which the spectrum was carried out by optical system. moreover, invention corresponding to claim 5 -- said spectrum -- it is the flying spot microscope according to claim 4 characterized by being the dichroic mirror which carries out the spectrum of the light from said sample which passed said parallel light conversion optical system as optical system, and became parallel light with the predetermined spectral characteristic.
[Procedure amendment 6]
[Document to be Amended] Specification
[Item(s) to be Amended] 0029
[Method of Amendment] Modification
[Proposed Amendment]
[0029]
[Function] Since according to invention corresponding to claim 1 the laser beam of single wavelength is irradiated at least at a sample, and the spectrum of the light from a sample is carried out by at least one grating and a photodetector can detect the light from the sample by which the spectrum was carried out, good detection of S/N can be performed.
[Procedure amendment 7]
[Document to be Amended] Specification
[Item(s) to be Amended] 0030
[Method of Amendment] Modification
[Proposed Amendment]
[0030] Since according to invention corresponding to claim 2 two or more waves of laser beams are irradiated at a sample, the fluorescence to which it comes from a sample is separated by the photometry separation means and it is detected by the photodetector, separation of still more various fluorescence wavelength can be performed compared with invention corresponding to claim 1. Furthermore, since the light which passed the confocal diaphragm according to parallel light conversion optical system is changed into parallel light according to invention corresponding to claim 3, there is no loss of the total quantity of light, incidence can be carried out to a photodetector, and a photodetector can be arranged freely, without receiving the constraint on optics.
[Procedure amendment 8]

[Document to be Amended] Specification
[Item(s) to be Amended] 0031
[Method of Amendment] Modification
[Proposed Amendment]
[0031] the light from a sample which according to invention corresponding to claim 4 irradiated the laser beam of single wavelength at least at the sample, and passed the confocal diaphragm -- parallel light conversion optical system -- parallel light -- changing -- a spectrum -- since a spectrum is carried out according to optical system and the photodetector detected the light from the sample by which the spectrum was carried out, the light from a sample can be led to a photodetector without a loss, and, moreover, it becomes cheap with it being small and cheap. Moreover, according to invention corresponding to claim 5, the spectrum of the light from the sample which passed parallel light conversion optical system with at least one dichroic mirror, and became parallel light can be carried out with the predetermined spectral characteristic.

[Translation done.]

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(74)【代理人】
【弁理士】
【氏名又は名称】鈴江 武彦

(57)【要約】
【目的】測光分離手段として波長依存性のあるフィルタ類を使用することなく、多重染色時の蛍光クロスオーバのないS／Nのよい蛍光検出の可能な走査型光学顕微鏡を得る。
【構成】少なくとも単一波長以上のレーザビームを出射し標本19に照射するレーザ光源手段(レーザ光源1，ビームエクスパンダ2，ダイクロイックミラー4，XY走査光学系5，瞳投影レンズ6，顕微鏡7)と、標本19より発せられる蛍光を測光用光路上に配置されたグレーティング9と単一以上の可変幅スリット(10，11，12)を経由してこのスリット10～12に該当する光検出器13，14，15に導く測光分離手段を具備したことを特徴とする走査型光学顕微鏡。

【特許請求の範囲】
【請求項1】少なくとも単一波長のレーザビームを走査して標本に照射するレーザ光源手段と、前記標本からの光を検出する検出光学系と、前記標本からの光を結像する結像光学系と、この結像光学系の焦点位置に配置された共焦点絞りと、この共焦点絞りを通過した蛍光を複数の波長に分ける少なくとも1個のグレーティングと、このグレーティングにより分光された前記標本からの光を検出する光検出器と、この光検出器に前記グレーティングからの導入される光の幅を可変可能な少なくとも1個のスリットと、を具備したことを特徴とする走査型光学顕微鏡。
【請求項2】前記レーザ光源手段は少なくとも二波長以上のレーザビームを出射し標本に照射することを特徴とする請求項1記載の走査型光学顕微鏡。
【請求項3】少なくとも単一波長のレーザビームを走査して標本に照射するレーザ光源手段と、前記標本からの光を検出する検出光学系と、前記標本からの光を結像する結像光学系と、この結像光学系の焦点位置に配置された共焦点絞りと、この共焦点絞りを通過した発散光を平行光線にするコリメート光学系と、このコリメート光学系の後方に配置され、所定の分光特性で前記標本からの蛍光を分光する少なくとも1個のダイクロイックミラーと、このダイクロイックミラーで分光された標本からの光を検出する光検出器と、を具備したことを特徴とする走査型光学顕微鏡。

【発明の詳細な説明】
【0001】
【産業上の利用分野】本発明は単一以上の蛍光検出光学系と蛍光観察光学系を具備した走査型光学顕微鏡に関する。
【0002】
【従来の技術】
＜第1従来例＞従来、蛍光観察が可能な走査型光学顕微鏡として、米国特許4997242号明細書に開示されており、これは単一波長のレーザ光源を用いて単一、あるいは二つの蛍光観察を行うもので、図7はその第1従来例を説明するための図である。図7に示すように、レーザ発振器24から射出されたレーザビームは、以下に述べる光学走査部材25により走査される。
【0003】すなわち、ビームスプリッタ23により反射され、第1ガルバノメータスキャナを構成するガルバノメータ46aと平面鏡35a、および凹面鏡42a，42bならびにガルバノメータスキャナを構成するガルバノメータ46bと平面鏡35bにより2次元走査したのち顕微鏡を通して標本上に照射される。これによって標本に発した蛍光は逆の光路をたどり、ビームスプリッタ32を通過したのち、単一蛍光時はフォトマルチプライヤ30へ、また二つの蛍光時はビームスプリッタ32により分光され、各々フォトマルチプライヤ30、フォトマルチプライヤ34によって検出される。なお、以上述べた構成以外に、接眼レンズ27、アイリス用ダイヤフラム31，33を備えている。
【0004】このような構成のものにおいて、図示しない標本からの蛍光は、接眼レンズ27、平面鏡35b、凹面鏡42a，42bを反射した後、平行光線となり、平面鏡35aで反射され、アイリス用ダイヤフラム31，33を通過してフォトマルチプライヤ30，34で検出される。
【0005】以上述べた第1従来例の結像光学系は、ダイヤフラム31，33から凹面鏡42aまでの光路を大きくとることによって、共焦点効果が得られる。対物レンズが焦点位置の時に標本からの蛍光は、凹面鏡42aからフォトマルチプライヤ34までの間で平行光線になるので、フォトマルチプライヤ34に導かれる光ビーム直径は、ダイヤフラム31，33の直径で決定される。
【0006】＜第2従来例＞図8に示すように、レーザ光源1からのレーザビームは、適宜なるビーム直径に拡大するための光学系であるビームエキスパンダ2を通り、ビーム直径を拡大した後、レーザ波長を選択するためのレーザラインフィルタ3でレーザ波長を選択してダイクロイックミラー4で反射され、ガルバノミラー等のX－Y走査光学系5でXY偏光され、瞳レンズ6、顕微鏡7を介してレーザビームは標本19上に照射され、標本19をビーム走査することになる。
【0007】これにより励起された標本19からの蛍光は、顕微鏡7からのダイクロイックミラー4に至る経路を戻り、ダイクロイックミラー4を通過した光は、ダイクロイックミラー64で分光され、一方は結像レンズ71を通り、共焦点絞り74を通って光検出器15で検出される。
【0008】同様に、他方はダイクロイックミラー65で分光され、結像レンズ72を通り、共焦点絞り75を通って光検出器14で検出され、またダイクロイックミラー65を通過した蛍光は、ミラー66で反射され、結像レンズ73を通り、共焦点絞り76を通って光検出器13で検出される。
【0009】以上述べた第2従来例は、結像レンズ71の焦点距離fと共焦点絞り74へ光検出器15ま

での距離lを適当に設定することにより、光検出器15の受光領域に標本19からの蛍光を導くことができる。同様に、結像レンズ72，73の焦点距離fと共焦点絞り75，76へ光検出器14，13までの距離lを適当に設定することにより、光検出器14，13の受光領域に標本19からの蛍光を導くことができる。
【0010】＜第3従来例＞図9は図8を以下のように構成したものである。すなわち、図8のダイクロイックミラー64，65と光検出器15，14の間にそれぞれ配設されている結像レンズ71，72と共焦点絞り74，75ならびにミラー66と光検出器13の間に配設されている結像レンズ73と共焦点絞り76を設けず、ダイクロイックミラー4と64の間に結像レンズ77と共焦点絞り78を設けたものである。
【0011】このように第3従来例のように構成にすると、図8と同様な機能が得られると共に、図8の従来例に比べて装置が簡単になり、コストダウンとなる。
＜第4従来例＞また、従来、蛍光検出が可能な走査型光学顕微鏡として、米国特許5127730号明細書に開示されており、これは複数の波長のレーザ光源を用いて、2つの蛍光を検出するもので、図10はその第4従来例を説明するための図である。レーザ光源50として、488nm，568nm，647nmのレーザ光を同時発振するKr－Arレーザ光源50を用いている。
【0012】レーザ光源50より発振された3つの波長のレーザ光51は、励起フィルタ52のデュアルバンドパスフィルタ52aで488nmと568nmの2つとなり、デュアルダイクロイックミラー54により図の下方の対物レンズ（図示せず）を介して蛍光標本55に導かれる。標本55から蛍光として発光された2種類の波長は、前記対物レンズ、デュアルダイクロイックミラー54を透過し反射鏡56で反射され、フィルタブロック57に導かれる。そして、フィルタブロック57に有する測光用ダイクロイックミラー57aにより、1つ1つの波長に分光されフィルタ57bおよび57cをそれぞれ介してフォトマルチプライヤ（PMT）58及び59により検出される。
【0013】このように、図10に示す走査型光学顕微鏡によれば、複数の波長を発振するマルチラインレーザ光源を組み合わせて、2重励起観察を行うことができる。
＜第5従来例＞一方、第5従来例には、3つの蛍光を検出する走査型光学顕微鏡が開示され、図11はこれを説明するための図である。レーザ光源160より発振された488nmと514nmのレーザビーム161は、エクスターナルフィルタ162により、どちらか1つの波長のみが透過される。
【0014】そして、ビームスプリッタ163により、図示下方に反射されXYスキャニングユニット164を通り、光学顕微鏡内の接眼レンズ166および対物レンズ167を通り標本165に集光される。この場合、標本165面はスキャニングユニット164で、標本165面を2次元に走査される。すると、標本165より発光した蛍光は、対物レンズ167、接眼レンズ166、スキャニングユニット164を通る。
【0015】そして、ビームスプリッタ163を透過し、2波長測光の場合はビームスプリッタ168により分光され、この一方はフォトマルチプライヤ174に導かれ、ビームスプリッタ168の他方の分光はビームスプリッタ169により分光され、この一方はフィルタ172を介してフォトマルチプライヤ173により検出され、ビームスプリッタ169の分光の他方はフィルタ170を介してフォトマルチプライヤ171により検出される。
【0016】このようにして標本165に適宜、照射されたレーザ光161からの蛍光はビームスプリッタ163を通過し、ビームスプリッタ168及び169により分光され、各々、フォトマルチプライヤ171，173，174によって検出される。
【0017】近年、蛍光観察においては、単染色のみならず、多重染色が多用されている。もとより蛍光染色は細胞、組織内の特定対象を可視化（特異性）する為に行う。故に多重染色時は各々の染色部位が明確な色の差、即ち蛍光波長の違いとして標識されなければならない。
【0018】ところで蛍光染色には極めて多種の方法があり、多重染色を行うと蛍光波長に部分的な重なり部分（クロスオーバ部分）が生じることがあり、図12はそれを示している。
【0019】
【発明が解決しようとする課題】前述した第1従来例では、凹面鏡42a，42bからダイアフラム31，33までの光路長を、共焦点効果を得るために、大きく取らねばならないので、装置が大型化する。さらに第1従来例を小型化するために、図7のミラー100a，100b，100cのように反射させねばならないが、これは光量をロスする原因になる。
【0020】図8の第2従来例では、各チャンネルに共焦点絞り74～76および結像レンズ15，14，13を必要とするので、装置が複雑になり、コスト高となる。図9の第3従来例では、ダイクロイックミラー64，65を配置し、3チャンネル構成となっているので、光検出器14は15よりも光路長が大きくなり、また光検出器13は14よりも光路長が大きくなる。従って、光検出器13，14の受光領域に

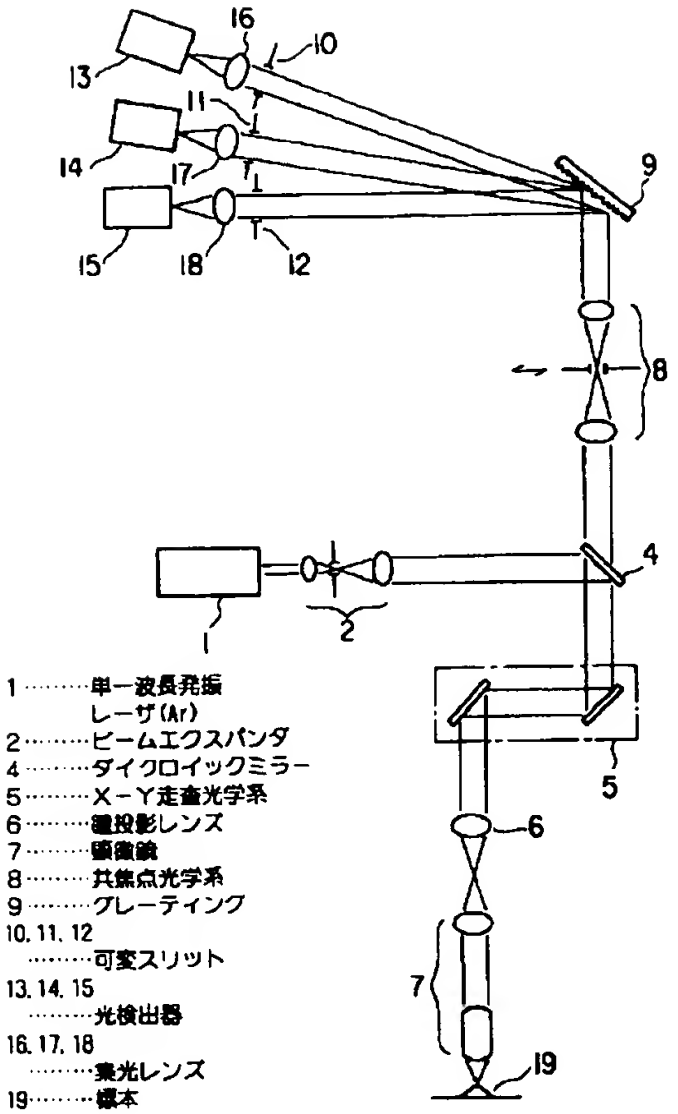
光ビームが完全に導かれなくなる。
【0021】図9の第3従来例では、光検出器15，14，13の受光領域にロスなく標本19からの蛍光を導くには、結像レンズ77の焦点距離fを大きくしなければならない。しかし、焦点距離fを大きくすると、装置が大型化するという欠点がある。
【0022】また図10の第4従来例または図11の第5従来例では、これら多重染色による複数の蛍光波長を分割する手段として、ビームスプリッタ(ダイクロイックミラー)54または163を使用し、更に検出する蛍光波長を限定する為に、様々な種類のシャープカットフィルタやバンドパスフィルタ52a，52b，52c、57a，57b，57cまたは168，169，170，172を使用する必要がある。これらは使用するレーザ波長や蛍光染色に合わせ、その都度準備する必要もある。
【0023】一般的に、これらの波長依存性のあるフィルタ類52a～52c、57a～57c、168～170，172は非線形性であり、蛍光波長のクロスオーバを取り除こうとすると、蛍光量のかなりの部分を検出する前に捨て去ることとなる。このことにより、検出のS／Nが落ちる。また染色の種類によっては蛍光波長のクロスオーバが大きいものも存在する。従って、前述した第4従来例または第5従来例では該検出そのものが不可能となる。
【0024】本発明の第1の目的は前記不具合を解消し、多重染色時の各々の蛍光波長を検出するための波長依存性のあるフィルタ類を使用することなく、S／Nの良い検出を行うことができる走査型光学顕微鏡を提供することにある。
【0025】本発明の第2の目的は前記不具合を解消し、標本を反射して得られる蛍光を光検出器にロスなく導くことができ、しかも小型で安価となる走査型光学顕微鏡を提供することにある。
【0026】
【課題を解決するための手段】前記目的を達成するため、請求項1に対応する発明は、少なくとも単一波長のレーザビームを走査して標本に照射するレーザ光源手段と、前記標本からの光を検出する検出光学系と、前記標本からの光を結像する結像光学系と、この結像光学系の焦点位置に配置された共焦点絞りと、この共焦点絞りを通過した蛍光を複数の波長に分ける少なくとも1個のグレーティングと、このグレーティングにより分光された前記標本からの光を検出する光検出器と、この光検出器に前記グレーティングからの導入される光の幅を可変可能な少なくとも1個のスリットと、を具備したことを特徴とする走査型光学顕微鏡である。
【0027】前記目的を達成するため、請求項2に対応する発明は、前記レーザ光源手段として少なくとも二波長以上のレーザビームを出射し標本に照射することを特徴とする請求項1記載の走査型光学顕微鏡である。
【0028】前記目的を達成するため、請求項3に対応する発明は、少なくとも単一波長のレーザビームを走査して標本に照射するレーザ光源手段と、前記標本からの光を検出する検出光学系と、前記標本からの光を結像する結像光学系と、この結像光学系の焦点位置に配置された共焦点絞りと、この共焦点絞りを通過した発散光を平行光線にするコリメート光学系と、このコリメート光学系の後方に配置され、所定の分光特性で前記標本からの蛍光を分光する少なくとも1個のダイクロイックミラーと、このダイクロイックミラーで分光された標本からの光を検出する光検出器を具備したことを特徴とする走査型光学顕微鏡である。
【0029】
【作用】請求項1に対応する発明によれば、単一波長のレーザビームを標本に照射し、標本からくる蛍光が測光分離手段により分離され、かつ光検出器によって検出されるので、S／Nが良く、蛍光波長の分離ができる。
【0030】請求項2に対応する発明によれば、二波長以上のレーザビームを標本に照射し、標本からくる蛍光が測光分離手段により分離され、光検出器によって検出されるので、請求項1に対応する発明に比べて更に多様な蛍光波長の分離ができる。
【0031】請求項3に対応する発明によれば、少なくとも1つのダイクロイックミラーで標本を反射して得られる蛍光を光検出器にロスなく導くことができ、しかも小型で安価となる。
【0032】
【実施例】以下、本発明の実施例について図面を参照して説明する。
＜第1実施例＞図1は本発明の走査型光学顕微鏡の第1実施例の光学系を示す図である。本実施例は、レーザ光源1は単一波長例えば488nmのレーザビームを出射し、標本19に照射する。レーザ光源1からのレーザ光は、後述するレーザ光源手段および測光分離手段に導かれる。レーザ光源手段はビームエクスパンダ2、ダイクロイックミラー4、X－Y走査光学系5、瞳投影レンズ6、顕微鏡7を順次介して標本19に導くように構成されている。
【0033】また測光分離手段は、標本19からの蛍光をダイクロイックミラー4にて分離したのち、共

焦点光学系8、グレーティング9、幅を変更可能なスリット10，11，12、集光レンズ16，17，18、光検出器13，14，15からなっている。
【0034】このような構成のものにおいて、標本19から発した蛍光は、顕微鏡7から共焦点光学系8を通過したのち、グレーティング9に至る。勿論この時、共焦点光学系8をバイパスさせることも可能である。グレーティング9に至った蛍光はその波長に合わせ、0次～n次光に分けられる。これらの各次光には各々スリット10，11，12、集光レンズ16，17，18、光検出器13，14，15が対応する。各々、スリット10，11，12の幅を変化させることで、検出する各々の蛍光波長範囲が変更可能となる。
【0035】以上述べた第1実施例によれば、測光分離手段としてダイクロイックミラー、シャープカットフィルタ、バンドパスフィルタ等の波長依存性のあるフィルタ類を使用することがないので、多重染色時の蛍光クロスオーバのないS／Nのよい検出が可能になる。
【0036】＜第2実施例＞図2は、本発明の第2実施例の光学系を示す図であり、前述の第1実施例のビームエクスパンダ2とダイクロイックミラー4の間の光路上に、新たにダイクロイックミラー22およびレーザラインフィルタ3を設け、さらにダイクロイックミラー22には、単一あるいは複数波長同時発振レーザ光源20からのレーザ光をビームエクスパンダ2に拡大して照射する構成ように構成されている。これ以外のダイクロイックミラー4で反射された後、検出器13，14，15に至るまでの構成は第1実施例と同一である。
【0037】レーザ光源20としては、488nm、568nmのAr—Krレーザ光源、351nmのArレーザ光源を組合わせたものを用いる。以上述べた第2実施例も、前述の第1実施例と同様な作用効果が得られる。すなわち、レーザ光源1，20からの二波長以上のレーザビームを標本19に照射し、標本19からくる蛍光がグレーティング9により分離され、光検出器13，14，15によって検出されるので、S／Nが良く、第1実施例に比べて更に多様な蛍光波長の分離ができる。
【0038】＜第3実施例＞図3は、本発明の第3実施例の光学系を示す図であり、前述の第1実施例のレーザ光源1を、例えば351nm，458nm，488nm，514．5nmのマルチラインArレーザ光源からなる複数波長同時発振レーザ光源21に変更し、ビームエクスパンダ2とダイクロイックミラー4の間に、レーザラインフィルタ3を設けたものであり、これ以外の構成は前述の第1実施例と同一である。
【0039】第3実施例によれば、レーザ光源21からの二波長以上のレーザビームを標本19に照射し、標本19からくる蛍光がグレーティング9により分離され、光検出器13，14，15によって検出されるので、S／Nが良く、第1実施例に比べて更に多様な蛍光波長の分離ができる。
【0040】＜第4実施例＞図4は、本発明の第4実施例の光学系を示す図であり、図1の実施例と異なる点は、以下のように構成したものである。すなわち、標本19からの反射光を集光する結像レンズ61と、結像レンズ61の結像位置に配置された共焦点絞り62と、この共焦点絞り62を通過する発散光（拡がり角をもつビーム）を平行光線にするコリメート光学系63と、このコリメート光学系63の後方に配置され、所定の分光特性で標本19からの蛍光を分光する2個のダイクロイックミラー64，65と、ダイクロイックミラー65の後方に配置され、ダイクロイックミラー65から得られる分光を反射して光検出器13に導くミラー66を設けたものである。
【0041】このような構成のものにおいて、コリメート光学系63により、共焦点絞り62を通過した光（拡散光）は平行光に変換される。従って、共焦点絞り62を通過した後の光を所定の波長毎に分光して異なる複数の波長の光をそれぞれの光検出器15，14ならびに13によって測光することができる。
【0042】この場合、共焦点絞り62からどのような距離に光検出器15，14，13を配置しても、測定光束はコリメート光学系63により平行光に変換されることから、全光量がロスなく、ダイクロイックミラー64，65、ミラー66を介して光検出器15，14ならびに13に入射する。従って、ダイクロイックミラー64，65、光検出器15，14ならびに13は、光学上の制約を受けることなく、自由に配置できる。
【0043】＜第5実施例＞図5は、本発明の第4実施例の光学系を示す図であり、図4のコリメート光学系63を、以下のようなコリメート光学系67としたものである。すなわち、コリメート光学系67は、片面が平面の凸レンズ、片面が球面上の凸レンズとし、かつこの凸レンズの平面側の面に図示しない蒸着膜にピンホールを形成したものである。
【0044】このように構成することにより、コリメート光学系67を通過した光ビームは平行光線になり、ダイクロイックミラー64，65で分光され、光検出器15，14ならびに13に受光領域から外れることなく導かれる。
【0045】コリメート光学系67は、共焦点絞りを兼ねているので、小型でかつ安価にできる。

＜第6実施例＞図6(a)は、本発明の第6実施例の光学系を示す図であり、図5の実施例のコリメート光学系67を、凸レンズ67aと凹レンズ67bの組み合わせたものとし、測光分離手段はグレーティング9で構成し、さらに光の幅を変更可能なスリット10，11，12、集光レンズ16，17，18、光検出器13，14，15から構成したものである。
【0046】このような構成のものにおいて、図示しない標本から発した蛍光は、図示しない顕微鏡、瞳投影レンズ、X－Y走査光学系、ダイクロイックミラーを通過し、結合レンズ61、共焦点絞り62、コリメート光学系67を通ってグレーティング9に至る。蛍光は、その波長に合わせ、0次～n次光に分けられる。これらの各次元には、各々スリット10～12、集光レンズ16～18、光検出器13～15が対応する。各々、スリット10～12の幅を変化させることで、検出する各々の蛍光波長範囲が変更可能となる。
【0047】第6実施例では、コリメート光学系67を、凸レンズ67aと凹レンズ67bの組み合わせたものとすることにより、目的のビーム直径にするのに小型化ができる。これは、図6(b)に示すように、コリメート光学系67として凸レンズのみで構成した場合に比べてである。なお、ビーム直径は、グレーティング9の格子間隔に対して十分に大きくする必要がある。
【0048】
【発明の効果】本発明によれば、測光分離手段として波長依存性のあるフィルタ類を使用することなく、多重染色時の蛍光クロスオーバのないS／Nのよい蛍光検出の可能な走査型光学顕微鏡を提供できる。また、本発明によれば、標本を反射して得られる蛍光を光検出器にロスなく導くことができ、しかも小型で安価となる走査型光学顕微鏡を提供できる。

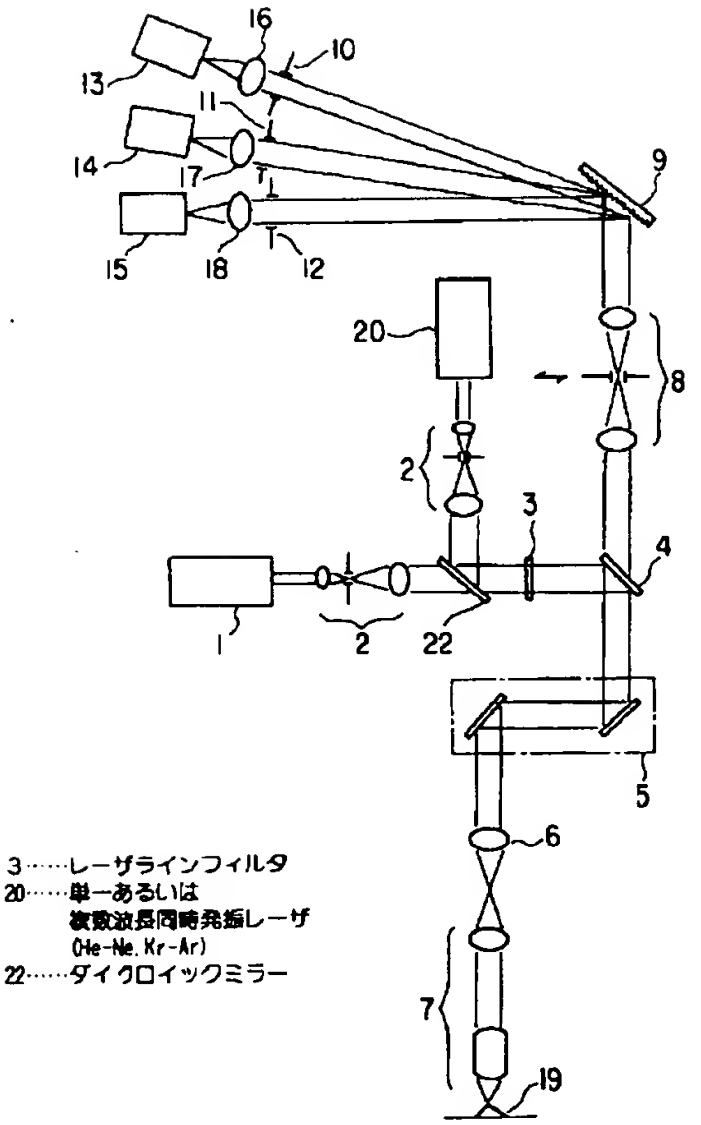
【図面の簡単な説明】
【図1】本発明の走査型光学顕微鏡の第1実施例を示す図。
【図2】本発明の走査型光学顕微鏡の第2実施例を示す図。
【図3】本発明の走査型光学顕微鏡の第3実施例を示す図。
【図4】本発明の走査型光学顕微鏡の第4実施例を示す図。
【図5】本発明の走査型光学顕微鏡の第5実施例を示す図。
【図6】本発明の走査型光学顕微鏡の第6実施例を示す図。
【図7】第1従来例を示す図。
【図8】第2従来例を示す図。
【図9】第3従来例を示す図。
【図10】第4従来例を示す図。
【図11】第5従来例を示す図。
【図12】蛍光のクロスオーバを示す図。
【符号の説明】
1…単一波長発振レーザ光源、2…ビームエクスパンダ、4…ダイクロイックミラー、5…XY走査光学系、6…瞳上投影レンズ、7…顕微鏡、8…共焦点光学系、9…グレーティング、10，11，12…可変スリット、13，14，15…光検出器、16，17，18…集光レンズ、19…標本、21，29…レーザ光源、61…結像レンズ、62…共焦点絞り、63，67…コリメータ光学系、64，65…ダイクロイックミラー、66…ミラー。

【図1】



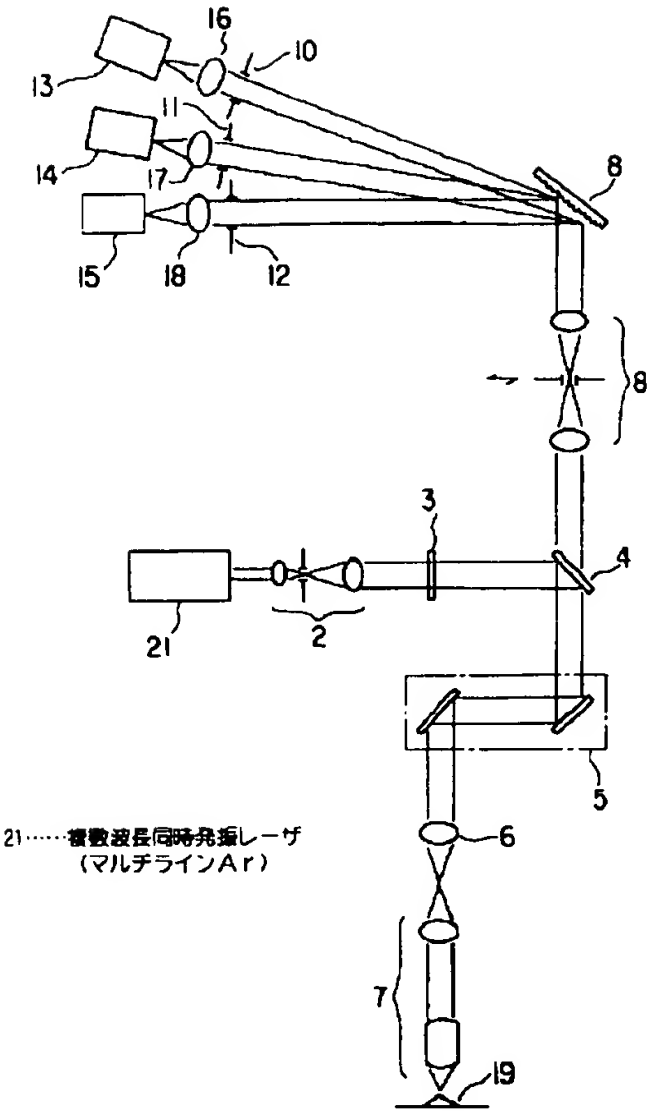
1……単一波長発振
レーザ(Ar)
2……ビームエクスパンダ
4……ダイクロイックミラー
5……X－Y走査光学系
6……縮投影レンズ
7……顕微鏡
8……共焦点光学系
9……グレーティング
10. 11. 12
……可変スリット
13. 14. 15
……光検出器
16. 17. 18
……集光レンズ
19……標本

【図2】

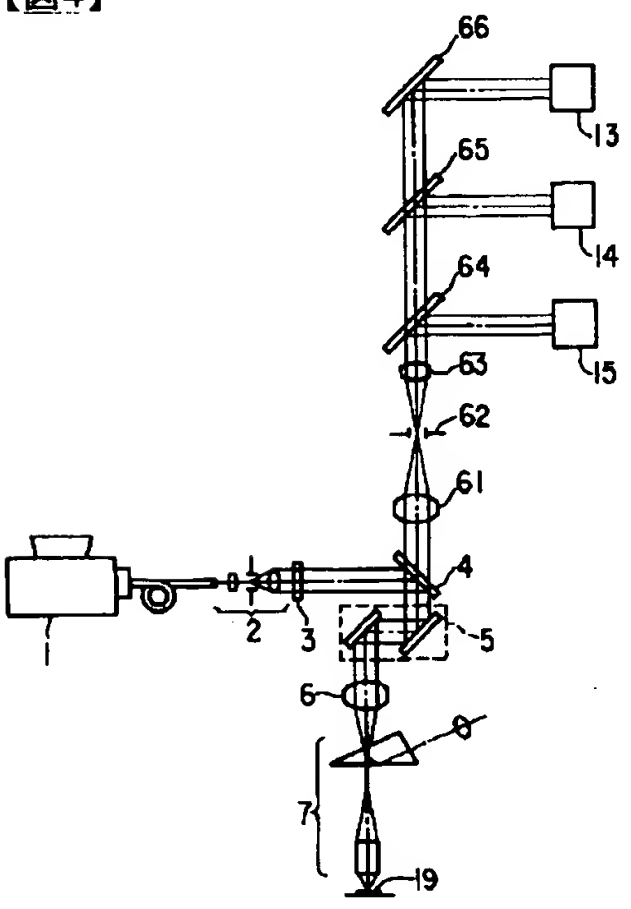


3……レーザラインフィルタ
20……単一あるいは
複数波長同時発振レーザ
(He-Ne, Kr-Ar)
22……ダイクロイックミラー

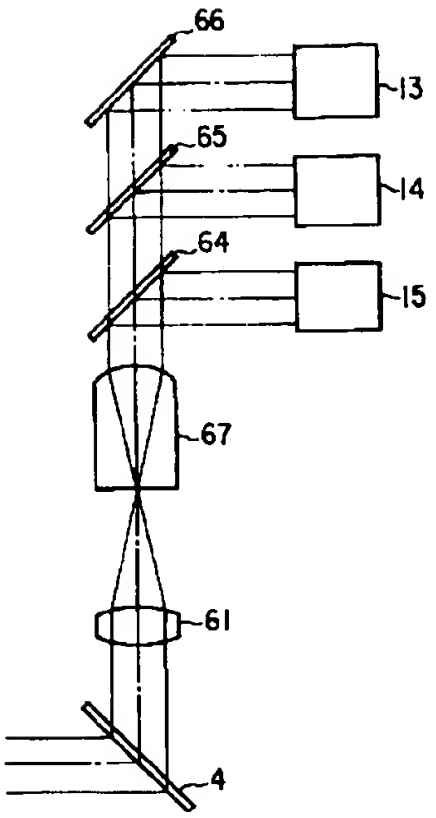
【図3】


16
10
13
11
14
17
8
15
18
12
8
3
4
21
2
5
21……複数波長同時発振レーザ
（マルチラインＡｒ）
6
7
19

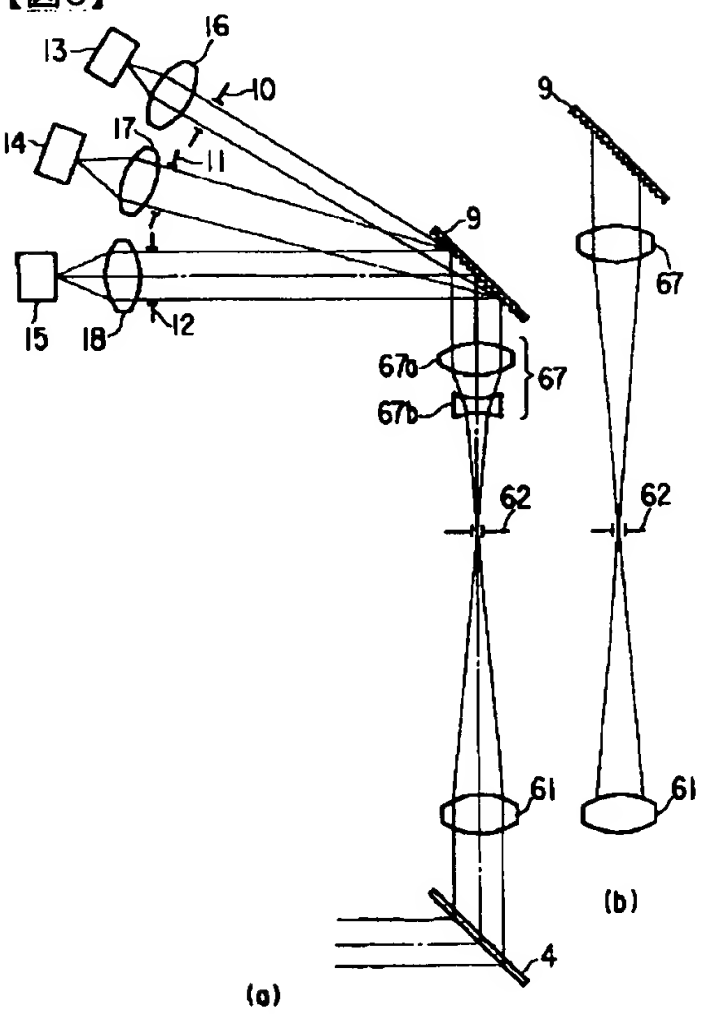
【図4】


66
13
65
14
64
15
63
62
61
4
2
3
5
6
7
19

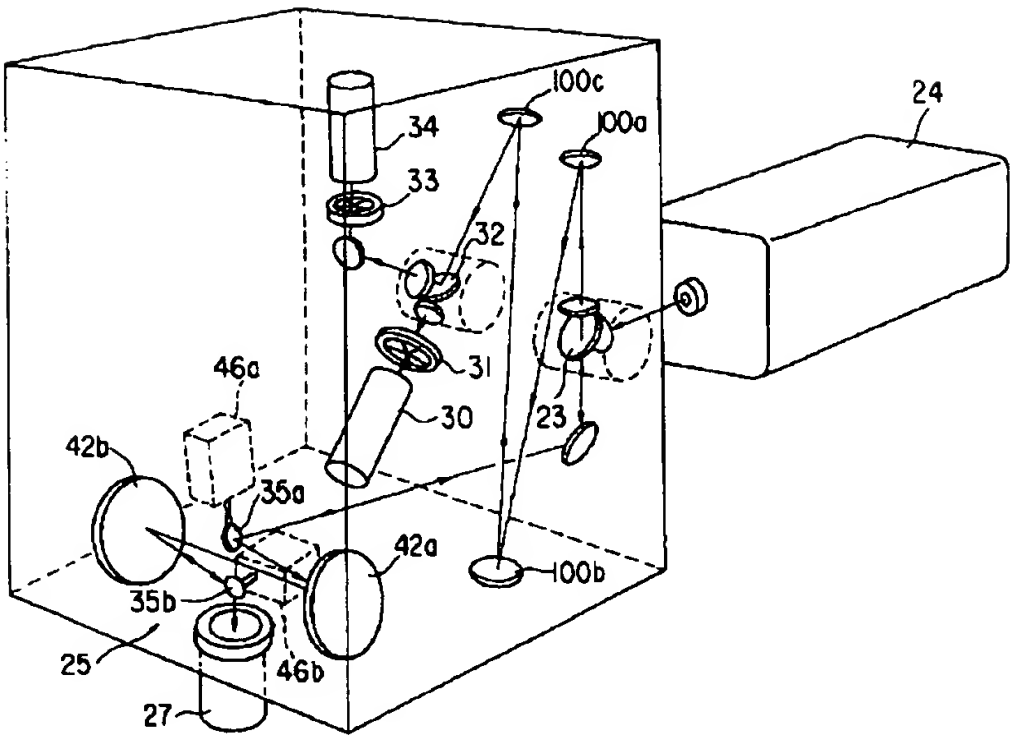
【図5】


66
13
65
14
64
15
67
61
4

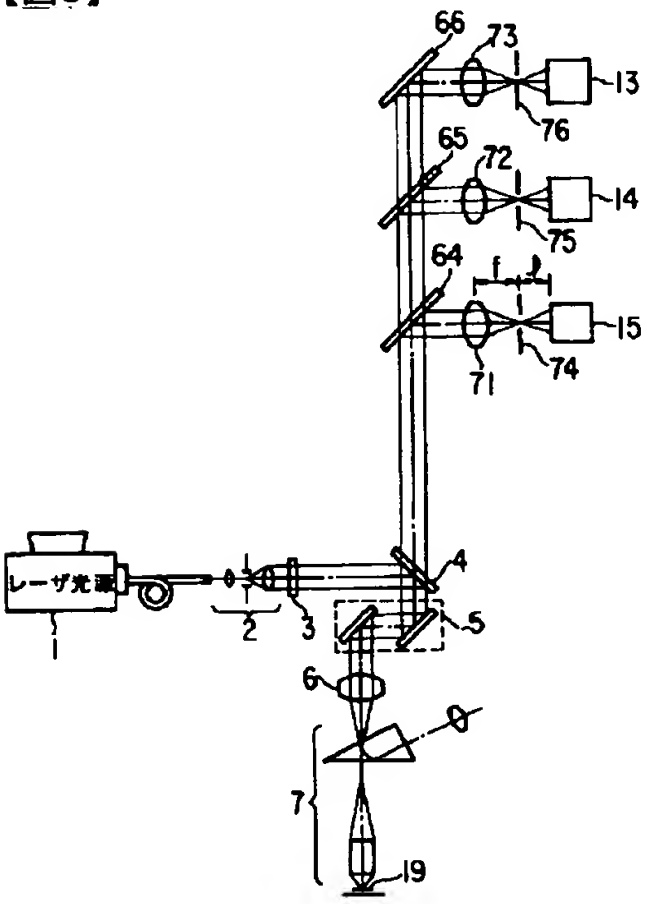
【図6】


16
13
10
17
14
11
9
12
15
18
67a
67
67b
62
61
4
(a)
9
67
62
61
(b)

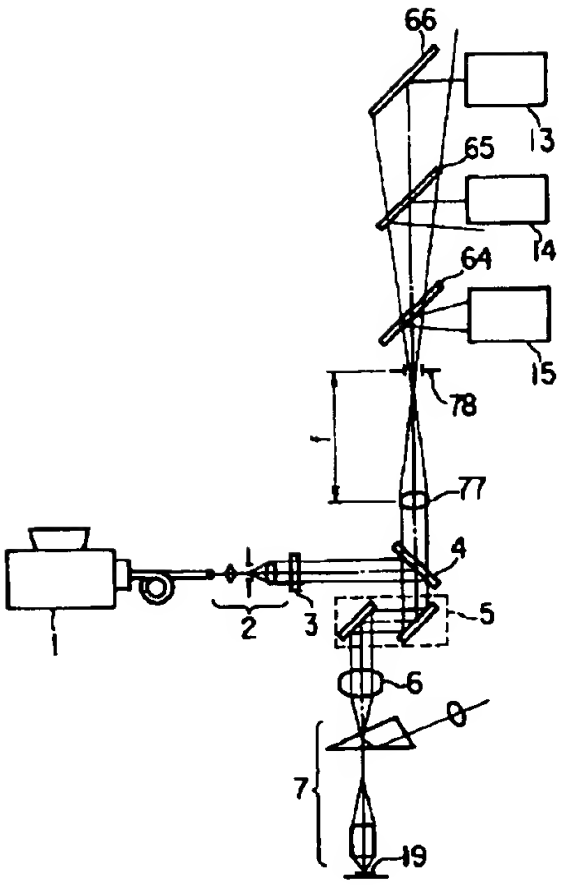
【図7】


100c
24
34
100a
33
32
31
46a
30
23
42b
35a
42a
100b
35b
25
46b
27

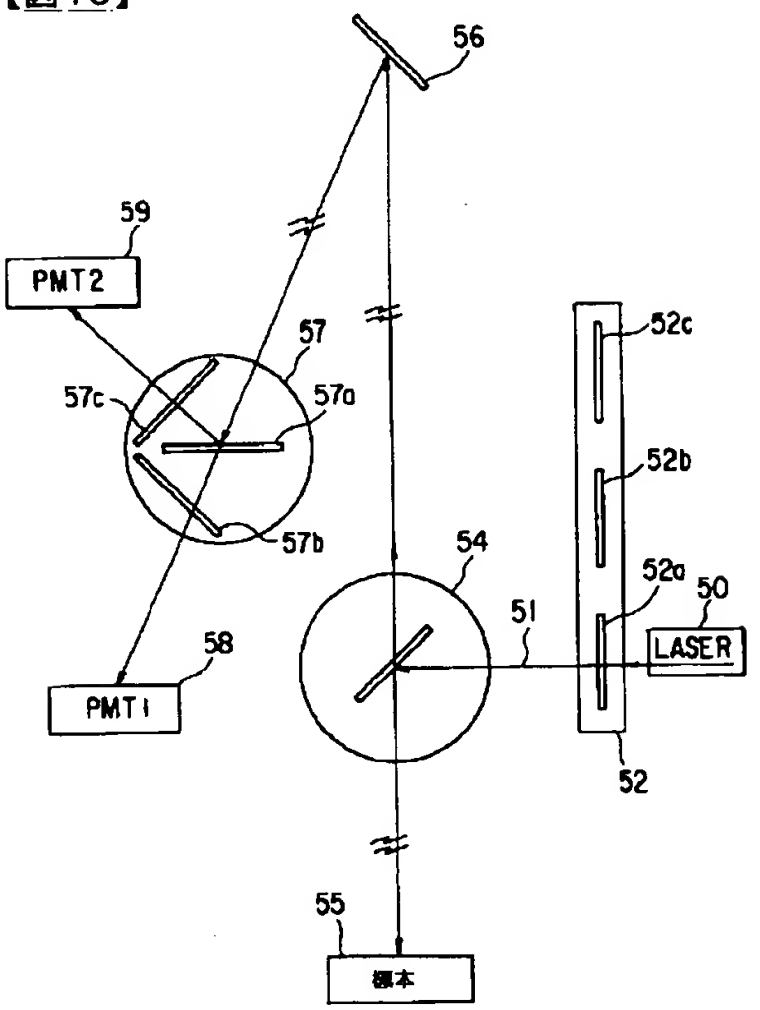
【図8】


66
73
13
76
65
72
14
75
64
15
74
71
レーザ光源
1
2
3
4
5
6
8
7
19

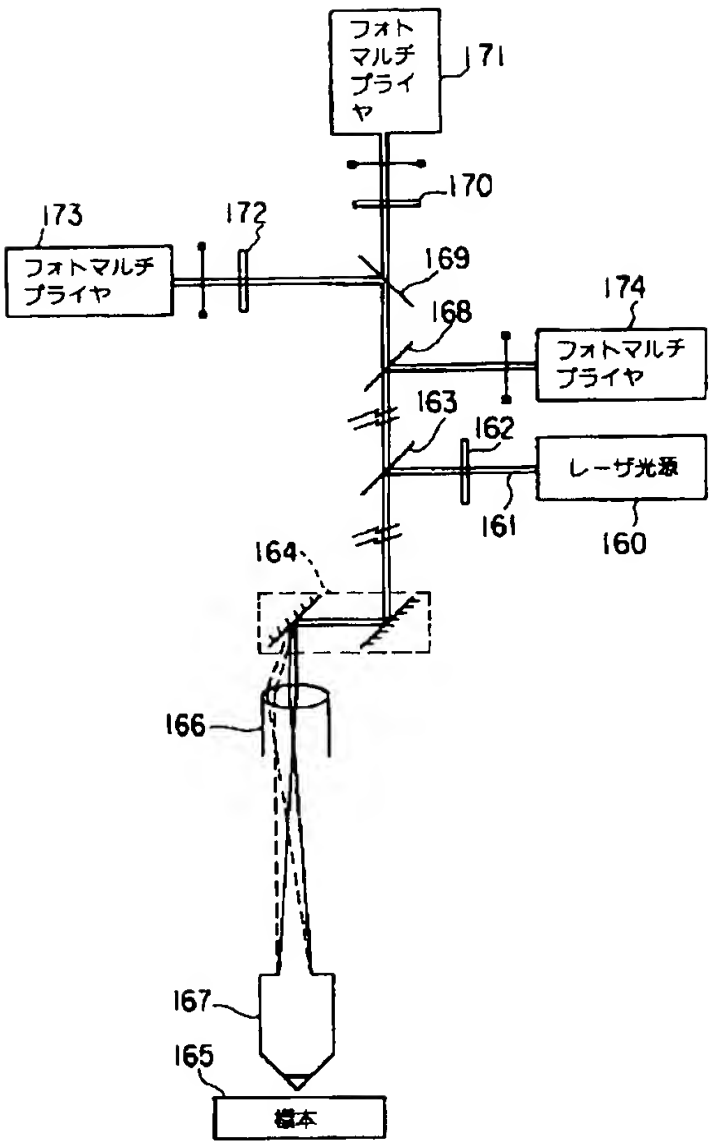
【図9】


66
13
65
14
64
15
78
77
4
5
6
8
7
19
1
2
3

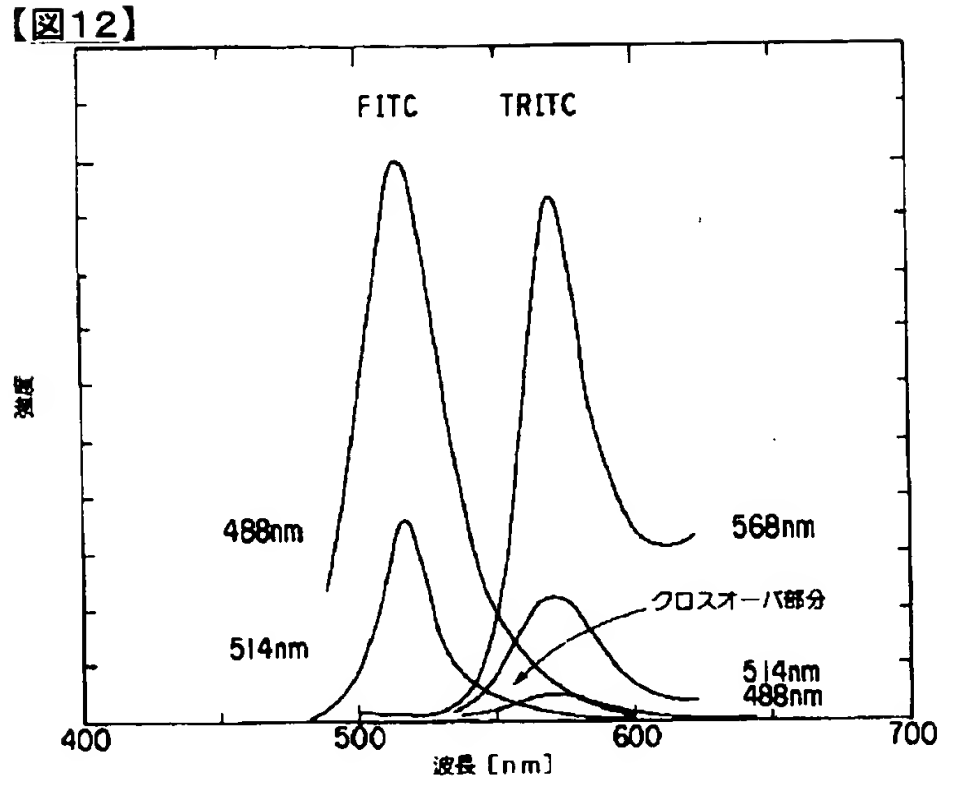
【図10】


56
59
PMT2
57
57c
57a
57b
58
PMT1
54
51
52c
52b
52a
50
LASER
52
55
標本

【図11】


フォトマルチプライヤ
71
170
173
172
フォトマルチプライヤ
169
174
168
フォトマルチプライヤ
163
162
レーザ光源
161
160
164
166
167
165
標本

【図12】


FITC
TRITC
488nm
514nm
568nm
クロスオーバ部分
514nm
488nm
400
500
600
700
波長 [nm]